DOCTORAL THESIS

The origins and appearance of sex differences in mental disorders

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The Origins and Appearance of Sex Differences in Mental Disorder

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Statement of Originality

I, Sarah I. Grime, declare that the work contained herein is my own unless otherwise cited. Information derived from the published and unpublished works of others has been acknowledged via referencing.

This thesis has not been submitted, in part or full, to another educational institution for the purpose of obtaining a degree qualification.

Sarah I. Grime

26 September, 2016

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Abstract

The biological underpinnings of sex differences were explored in relation to male- and female-specific functioning in typical development as a means of understanding how these factors may inform similar sex-based variation in atypical development. The influence of genetic and hormonal factors was explored in relation to sex-specific brain development and the subsequent sex-based variation in behaviour, including intelligence, social characteristics, and personality traits. Discussion then addressed sex differences in the symptom manifestation of mental disorder (i.e., depression and anxiety) and developmental disorder with a particular focus on Autism Spectrum Disorder. An exploration of the methodological issues encountered in researching sex-specific behavioural profiles in autism is presented highlighting the need for incorporating sex as a variable in future studies of ASD to better inform the diagnosis and treatment of females.

Keywords: Autism Spectrum Disorder, sex-differences, gender-differences, methodological issues

Introduction

Sex-based variation between males and females has been observed across the human lifespan in a variety of domains including morphology, physiology, psychology, and behaviour. These domains have been explored from varied medical, psychiatric and social perspectives in order to better understand the influence of sex-based factors on male- and female-specific experiences of heath and illness. Despite a growing appreciation for how sex-based variation between men and women contributes to disparity in health (e.g., developmental trajectories and cognitive ability) and disease (e.g., disease susceptibility, treatment responsiveness) there appears to be a poor understanding of the biological underpinnings for sex differences.

Explorations of sex-based variation have been confounded by terminological confusion between the terms 'sex' and 'gender', which has impeded the exploration of how biological (sex-based) and social (gender-based) factors impact human functioning (Afifi, 2007). A clear delineation of these terms is necessary in order to facilitate the exploration and clear understanding of how biological mechanisms and developmental processes contribute to the differential experiences of men and women in relation to health and illness.

Research has begun to assess how biological (such as genetics and hormones) may be responsible (in part) for the sex-based differences in brain structure and function that lead to variation in behaviour between men and women. In recent decades, medical and technological advances have also afforded greater understanding of those biological mechanisms that underlie disorder and how those mechanisms may act to influence the course and potential outcome of psychopathology. It has also become increasingly apparent that an individual's biological sex exerts an influence on normal psychological functioning across the lifespan and that biological sex is an important factor to consider when assessing, diagnosing, and treating mental illness (Wizemann, & Pardue, 2001). The issue of sex-disparity is pertinent to research concerning Autism Spectrum Disorder (ASD) where a well-recognised sex-based disparity in

prevalence rates (favouring males) has resulted in the suggestion that underlying biological mechanisms may be contributing to differential symptom manifestation between males and females with ASD (Rivet, & Matson, 2011a). However, investigations exploring a female-specific ASD profile have been encumbered by a number of methodological limitations that have made robust interpretations of behavioural findings difficult (Koenig & Tsatsanis, 2005; Mandy et al., 2012; Rivet, & Matson, 2011a).

The focus of this thesis is on how sex-based differences, as opposed to sex-based similarities, can inform further research direction and facilitate a better understanding of the significance of biological factors in understanding the male and females experience of health and disorder. *Chapter 1* provides an overview of the evolutionary significance of separate sexes (i.e., male versus female). It briefly explores the origins and evolution of differentiated sex-based entities and the advantages and disadvantages, from the perspective of sexual reproduction, of maintaining separate sexes. The chapter then introduces sexual dimorphism as the variation of characteristic between males and females and outlines the conditions under which sexual dimorphic phenotypes arose before moving into exploring the impact of male-female variation in the human species.

Chapter 2 presents findings to support clear distinction between the terms “sex” and “gender” as biological and social constructs respectively. That distinction is foundational to subsequent explorations (in this thesis) of the biological factors that give rise to sex-based differences in morphology, physiology, psychology and behaviour as independent of the social factors that influence the manner in which men and women differentially respond to situations as a result of socially-derived expectations imposed upon them by virtue of their biological sex. The discussion then extends to genetic and hormonal factors as causal biological mechanisms to the development of sexual dimorphisms between males and females.

Chapter 3 explores the development of the sexually dimorphic brain and the biological underpinnings (i.e., hormonal factors) that give rise to sex-based variation in the structure and neurophysiology of the human brain. An overview of the neuroimaging techniques used to investigate sex-based variation in male and female brains is presented before this chapter discusses the structural differences that have been most reliably observed through neuroimaging studies.

Chapter 4 of this thesis draws on the information discussed in the previous two chapters to explore the biological factors that promote the development of sex differences in behaviour. In addition to biological contributions, this discussion also introduces and explores socio-cultural (i.e., gender-based) factors as influential in the development and persistence of sex differences in behaviour. This chapter discusses sex-based behavioural variation between men and women in relation to three key areas: (i) general intelligence and cognitive abilities (discussed primarily as a demonstration of how structural brain differences translate into functional or behavioural differences), (ii) social characteristics (which focuses on play and activity interests as arising from social expectations derived from perceptions of biological sex), and (iii) personality traits (which explores aggression as an evolutionary-derived behaviour and an enduring characteristic of early developmental biological factors that is also influenced by social learning process).

Where the previous chapters have explored sex differences in various factors related to typical development, *Chapter 5* expands the exploration of this theme to the development of mental disorder. This chapter presents findings from research investigating depression and anxiety as these two disorders have shown consistent sex disparity in a number of factors, including prevalence, disorder onset, symptom manifestation, and treatment responsiveness. Chapter 5 highlights the importance of considering sex and gender as important and

interrelated variables that should be considered when assessing, diagnosing and treating these disorders.

Finally, *Chapter 6* addresses research investigating sex-based variation in neurodevelopmental disorder, with specific reference to Autism Spectrum Disorder (ASD). This chapter begins by providing a general overview of ASD symptomology, prevalence and the possible biological underpinning of the condition, before addressing the key methodological issues that confound investigations aimed at exploring sex-based variation in the behavioural manifestation of ASD symptoms between males and females. These limitations have been explored in relation to 30 investigative studies which reported phenotypic differences between males and females with ASD. This discussion highlights the importance of addressing methodological variation between studies from which inferences of a female-specific ASD profile. The focus of this chapter then moves onto an exploration of those biological factors implicated in the development of sex-based variation.

Chapter One: Evolution of Sexual Dimorphism

1.1: Origin of Two Sexes

Males and females are distinguished primarily by the presence of different gametes (i.e., reproductive cells capable of uniting with other reproductive cells). Sexually dimorphic gametes (i.e., existing or occurring in two distinct forms) are the evolutionary product of different mating types (molecular mechanisms that regulate sexual compatibility in sexual reproduction by determining which cells fuse together: Billiard, Lopez-Villavicencio, Hood, & Giraud, 2012; Kronstad & Staben, 1997) in sexually reproducing eukaryotes (organisms whose cells contain a nucleus and other organelles enclosed within membranes: Lehtonen & Parker, 2014; Raven & Johnson, 1986).

The first documented instance of separate sexes is thought to have been the *Funisia dorothea (F. dorothea)* species, estimated to have existed 565 million years ago (Droser & Gehling, 2008). Droser and Gehling (2008) described tubular organisms that were commonly assembled in densely packed groups of individuals (i.e., via a pattern of propagation that often accompanied sexual reproduction and which increased reproductive success by reducing gamete wastage) on the seafloor (see Figure 1, page 15 for a reconstruction of *F. dorothea* in life). It was first thought, because of the close proximity of individuals within the group, that *F. dorothea* reproduced asexually via budding, a process during which new individuals form as outgrowths of the bodies of a parent organism before breaking away to function independently of that parent body (Purves, Sadava, Orians, & Heller, 2004). However, this opinion was later questioned by researchers such as Droser and Gehling (2008), who observed that individuals within spatially clustered groups of *F. dorothea* were at similar growth stages, supporting sexual reproduction as the mechanism for this species' proliferation (Butterfield, 2000).

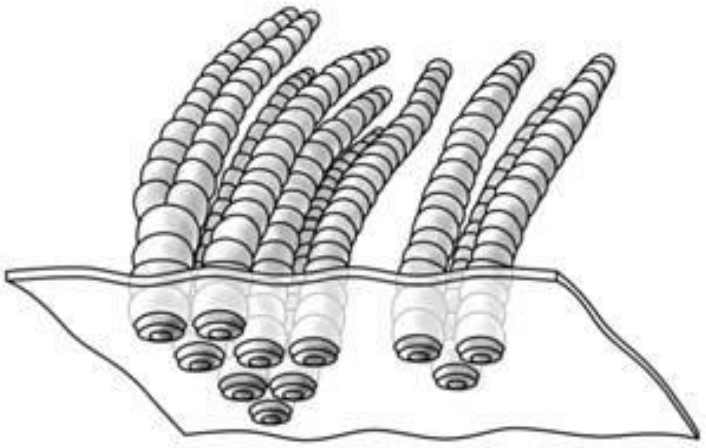

Figure 1: Reconstruction of F. dorothea. Sourced from "Synchronous Aggregate Growth in an Abundant New Ediacaran Tubular Organism," by M. L. Droser and J. G. Gehling, 2008, Science, 319, p. 1661.

Interestingly, the presence of separate sexes does not necessarily denote differentiation into separate sex-based entities, as evidenced by the fact that researchers have identified many eukaryotes that are neither male nor female (Xu & Yang, 2013). The classification of organisms as either male or female is based on the presence of different gametes and this differentiation results from the process of anisogamy. Anisogamy refers to a form of sexual reproduction which involves the fusion of two dissimilar gametes that can vary in relation to size or form (Yang, 2010). Bachtrog et al. (2014) suggested that, when one gamete is small and mobile and the other large and immobile, the fitness of both partners in the reproductive process is increased. Broadly defined, fitness in this context refers to an organism's ability to survive in its environment and reproduce successfully (Orr, 2009; Raven & Johnson, 1986). The fitness of both reproductive partners is increased when the differentiated gametes function cooperatively by fulfilling separate and distinct roles. In order for fertilisation to take place, each contributing gamete must have different but complimentary characteristics. First, one gamete must be transported to the other gamete, often across a distance, necessitating *mobility*. Second, this transportation process is risky in

that it may be lengthy and require energy, favouring a *smaller sized* gamete which would require less energy. Third, the risk of this smaller, mobile gamete not reaching the other may lead to its occurrence in greater *numbers*. In contrast, the larger and immobile gamete, due to being stationary, commits energy resources to the developing zygote to ensure its survival (Bachtrog et al., 2014; Yang, 2010). In anisogamous species, females produce fewer but larger immobile gametes (referred to as ovum), whereas males produce many smaller and more mobile gametes (referred to as sperm) that fuse to produce a diploid zygote (i.e., a eukaryotic cell formed by a fertilisation event between two gametes: Jones & Lopez, 2014) where the zygote's genome consists of DNA derived from each gamete (Barton & Charlesworth, 1998; Geng, De Hoff, & Umen, 2014). The development and maintenance of gamete sexual dimorphism is of evolutionary significance. It is believed that anisogamy most likely evolved from the production of morphologically identical gametes (i.e., isogamy) as a means of maximising diversity among offspring (Whitfield, 2004; Xu & Yang, 2013). Researchers (e.g., Bulmer & Parker, 2002 and Lehtonen & Parker, 2014) have hypothesised that anisogamy has persisted over time via the process of disruptive selection, which refers to the changes in population genetics that occur when extreme phenotypes demonstrate a fitness (i.e., the tendency to leave behind more offspring that are capable of reproducing than competing individuals do, Raven & Johnson, 1986) advantage over intermediate traits (Rueffler, Van Dooren, Leimar, & Abrams, 2006). Extreme phenotypes are selected and maintained because they offer adaptive value to the population of organisms (Bulmer & Parker, 2002; Rueffler et al., 2006).

Given its complexity, sexual reproduction is a costly (i.e., resource and energy intensive) process for a population and such a disadvantage would therefore promote the persistence of asexual reproduction. As asexual reproduction does not require the genetic recombination of two separate gametes, asexual individuals can produce twice as many

offspring as sexual individuals. Asexual reproduction is considerably less costly in comparison to sexual reproduction (Archetti, 2004). From an evolutionary perspective however, sexual reproduction also offers a number of benefits to a population (outlined in Table 1, page 18), which far outweigh the associated costs and therefore promoting the continuation of sexual dimorphism.

Table 1

Advantages and Disadvantages of Sexual Dimorphism

Factor	Advantages of Sexual Dimorphism	Disadvantages of Sexual Dimorphism
Gamete Production	1. Production of larger gametes provides a zygote with more resources, thereby increasing its chances of survival (Bachtrog et al., 2014; Whitfield, 2004).	1. The production of larger gametes (in an adult female) requires a greater expenditure of energy and time (Lehtonen & Parker, 2014).
Genetic Recombination	2. Genetic recombination produces unique offspring in each successive generation (Barton & Charlesworth, 1998) and: a. Allows faster adaptation to fluctuating environments (He, Yu, Ruan, & Yao, 2003) by promoting favourable allele combinations that are selected for by evolutionary forces (Roze & Otto, 2011). b. Enables a population to defend itself against parasites by ensuring constant change in individual variability (Geary, 1998; 98). c. Promotes resistance from the pressures of competing populations (Feigel, Englander, & Engel, 2009) by preventing gene flow between inappropriate populations and species (Snook, Gidaszewski, Chapman, & Simmons, 2013).	2. Genetic recombination destroys favourable combinations of alleles (Becks & Agrawal, 2010; Goddard, Godfray, & Burt, 2005).
Sexual Reproduction	3. Promotes cooperation through preferential interaction whereby each participating individual (who is willing and able to recognise other cooperating individuals) provides a fitness advantage to the other reproductive partner and resulting progeny (Feigel et al., 2009; Pizzari & Gardner, 2012).	3. Sex implies a two-fold cost, meaning that sexually reproducing females can only produce half the number of daughters than an asexually reproducing female (Feigel et al., 2009; West, Lively, & Read, 2001). 4. Energy and time costs associated with sexual reproduction include the finding and courting of potential mates, the increased risk of predation while engaging in these necessary activities, and the risk of contracting sexually transmitted diseases (Billiard et al., 2012; Roze & Otto, 2011).

Sexual dimorphism extends beyond mechanisms of sexual reproduction to include various morphological (e.g., body size and body composition), physiological (e.g., metabolism, brain function) and behavioural (e.g. reproductive/courting behaviours, communication and signalling behaviours) characteristics or traits, which can be observed between the sexes across a range of species (Dean & Mank, 2014; Snook et al., 2013). This differentiation between males and females has been primarily attributed to evolutionary selection forces that, over time, have promoted the development and maintenance of sex-specific phenotypes that aid in both survival and reproduction (Poissant, Wilson, & Coltman, 2009).

Sex-specific phenotypic differences are considered to have evolved primarily under the influence of sexual selection, which is broadly defined as those processes that act on an individual's ability to successfully copulate with a mate (Snook et al., 2013). Traits that (i) enhance an individual's ability to attract mates (e.g., ornamental characteristics such as bright plumage), (ii) benefit an individual when engaging in combat with rivals (e.g., weaponry), and (iii) deter opponents from engaging in combat due to the risk of injury (e.g., greater physical stature), provide an advantage over other same-sex members of the population in securing mates and these traits will therefore be selected for, or promoted, within a population (Hunt, Breuker, Sadowski, & Moore, 2008; McPherson & Chenoweth, 2012). Sexual dimorphism has also been considered as form of adaptation to sex-specific niche divergence. The term 'niche' refers to those environmental factors that influence the growth, survival and reproduction of a species; niche divergence is the process by which competing species use the environment in different ways to enhance their coexistence (Molles, 2013). Niche divergence that results from ecological competition between the sexes may promote the maintenance of sexual dimorphisms that arose from sexual selection (Shine, 1989; Slatkin, 1984). Sexual dimorphisms in body size between males and females in a species may, for example, reflect differing nutritional needs related to reproduction (e.g. gestation and

offspring rearing in females). In specific terms, sexual dimorphism is necessitated through reproductive competition and changing ecological conditions, two interacting pressures that impact a species' capacity to survive.

1.2: Exploring the Influence of Sexual Dimorphism in Humans

Sexual dimorphism in the human species involves multiple forms of differentiation between males and females including morphological, physiological and behavioural differences across the lifespan (McPherson & Chenoweth, 2012; Wizemann, & Pardue, 2001). From early development, males and females vary in relation to morphology, physiology and behaviour with differentiation of the gonads in the embryonic stage being the major determining factor for more distinguishable sexually dimorphic characteristics at puberty (the life stage during which the individual becomes sexually mature and capable of reproduction) (Geary, 1998). With the onset of puberty, the development of more distinct sexual dimorphisms occurs and is primarily attributable to sex-specific biological mechanisms (Wells, 2007).

Male-female variation has most often been examined in relation to how underlying biological factors might impact on physiological performance and the cognitive and emotional aspects of psychological functioning (Cosgrove, Mazure, & Staley, 2007; Johnson, 2001; Kanaan et al., 2012; Sacher, Neumann, Okon-Singer, Gotowiec, & Villringer, 2013; Whittle, Yücel, Yap, & Allen, 2011). However, biologically-based differences between men and women are important in contributing to individual variations, acting as significant determinants of health and disease (Pankevich, Wizemann, & Altevogt, 2011), and facilitating a greater understanding of male- and female-specific patterns of illness capable of improving treatment and care following injury (Wizemann, & Pardue, 2001). A number of human disease states show sex bias in terms of prevalence, symptom manifestation and severity (Pankevich et al., 2011; Stone et al., 2004). Further, some recent studies have sought to expand the exploration of sex-based contributions to mental illness by determining the biological influences and causes of mental disorders and developmental disabilities (Cosgrove et al., 2007; Wizemann, & Pardue, 2001). Sex-based differences observed in regular and irregular psychological

functioning in men and women also have implications in understanding disease prevalence and the clinical outcomes to treatment and intervention in males versus females. This thesis presents a systematic exploration of sex differences in males versus females and aims to link those differences to the biological factors that underlie them. The incorporated discussion differentiates between well supported findings, such as those explored in relation to typical development and well researched mental disorder, versus emerging trends, such as those seen in neurodevelopmental disability.

Chapter Two: Sex and Gender

2.1: Terminological Confusion in the Study of Sexual Dimorphism

Research investigating the influence of sex-linked factors on development, health and illness is often complicated by inconsistent and ambiguous reference to the terms '*sex*' and '*gender*' in the biomedical and psychiatric literature (Afifi, 2007). From a definitional perspective the term '*sex*' is used to distinguish between the biologically-defined groups of male and female, whereas '*gender*' is used to classify individuals based on personal attributions governed by social standards (Halpern, 2012; Johnson & Repta, 2012; Rutter, Caspi, & Moffitt, 2003). The term '*gender*' however, has also been used to refer to those psychological, cognitive and behavioural attributes that vary between males and females, despite the biological associations of these factors (Eckel et al., 2008; Halpern, 2012). Subsequently, these terms have been used interchangeably to describe a range of differences between males and females.

In this thesis, the term '*sex*' will encompass the biologically-determined characteristics of an individual, including genetic, hormonal, morphological (also referred to as structural), physiological (also referred to as functional), behavioural and psychological characteristics (Lenroot & Giedd, 2010; Ober, Loisel, & Gilad, 2008; Pankevich et al., 2011). The terms '*sex-based*' and '*sex-related*' will be used to refer to those characteristics that have biological associations, such as cognitive abilities, personality characteristics, and behaviour. Further, each factor will be explored in relation to those biological associations and the differential impact they have on phenotypic development in males and females (Halpern, 2012). Based on these biological factors, sex-based differences are those characteristics found to be reliably different between males and females (Eckel et al., 2008). There are a number of considerations to be taken into account when exploring sex differences in order to enhance understanding of the ways in which biological variable differentially impact on functioning in

males versus females. First, biological factors do not represent dichotomous variables, in which a characteristic would be considered to exist solely in a male or female. Instead, biologically-based characteristics exist along a continuum where a particular characteristic can be considered to be more common in one of the two sexes (Eckel et al., 2008; Hines, 2002). That is, both sexes can display a particular characteristic, but in one sex it is more pronounced or occurs more often than in the other sex. The possible extent of sex differences has been described by Eckel et al, (2008) who suggested that a characteristic may differ between the sexes in terms of its mean, variance, percentile value, ration, incidence, and timing. Second, biological factors are developmentally influenced across the lifespan and, while both sexes may display a particular characteristic, it could be more likely to occur at different developmental stages (e.g., prenatally, infancy, childhood, adolescence and adulthood) (Eckel et al., 2008; Ober et al., 2008). Third, pre-existing sex-specific factors are capable of influencing one or more biological characteristic by acting as a source of variability in one sex but not the other (Pankevich et al., 2011). An example of such a sex-specific factor is the oestrus cycle of females. Fluctuating hormones throughout a woman's oestrus cycle have been shown to impact on such factors as cognitive and motor performance (Hampson, 1990**;** Maki, Rich, & Rosenbaum, 2002). Overall, these factors contribute significantly to variation between the sexes as well as within the sexes and the study of sex differences should consider the impacts of each one (Blakemore, Berenbaum, & Liben, 2013; Johnson & Repta, 2012). Despite these cautions, sex-based biological differences constitute the most fundamental sexual dimorphisms between males and females and will be the focus of this thesis.

In extending the discussion in this thesis to '*gender'*, this term encompasses the culturally- and socially-determined expectations of how males and females behave. Therefore, this construct incorporates such factors as gender identity, gender expectations, and gender

roles, as well as the individual factors of expressing personality, coping ability, treatment-seeking, and symptom-reporting behaviours that arise from social and cultural expectations (Afifi, 2007). Gender is considered to relate to how individuals identify themselves within a culture or society as well as how they are perceived by others (Archer & Lloyd, 2002). Further, as Bekker and Mens-Verhulst (2007) propose, specific gender characteristics can be fluid and not easily indicative of strict male versus female differentiation, with some dissimilarities (e.g., gender identity) occurring between individuals of the same sex, and similarities (e.g., interests and activity preferences) being evident in people of the opposite sex. Factors relating to gender are influenced by sex-based biological differences between men and women as well as their environment and experiences (Pankevich et al., 2011).

In seeking to understand the central differences between the constructs of sex and gender, it is important to acknowledge that biological and socio-cultural influences are not mutually exclusive, but rather interact to contribute to an individual's overall experience of health and illness (Rieker & Bird, 2005; Rutter et al., 2003). Researchers (e.g., Afifi, 2007) have suggested that, due to this inter-connection, continued research into the impacts of biologically-based differences between men and women should not occur at the exclusion of any gender-based variation between men and women by virtue of their sex. In other words, the study of sex-based differences should provide an opportunity to better understand how biological factors interact with social and cultural factors and be used to guide the development of sex-specific strategies for the management of health, disease and mental disorder (Ngun, Ghahramani, Sánchez, Bocklandt, & Vilain, 2011; Pankevich et al., 2011).

Human sexual dimorphism begins early in development and there are two overarching biological mechanisms that contribute to differences between males and females: *sex determination* and *sex differentiation*. Each of these mechanisms governs a number of processes that impact a developing individual and contribute to differences in morphology,

physiology and behaviour across the lifespan, which will be discussed in more detail in Section 2.2 (pages 27-38) and Section 2.3 (page 39-43) below.

2.2: The Influence of Genetics on Human Sexual Dimorphism

Sex determination occurs at the genetic level and concerns the inheritance of genetic material from parents and the expression of that material in physical structure and functioning. The collection of genes (broadly described as molecular units of heredity that may influence the expression of characteristics, Brooker, 2005) an individual inherits is referred to as its "genotype", which acts as the set of instructions for the phenotypic (i.e., observable traits of an organism, Cummings, 2014) development of the individual. Males and females have largely similar genomes and many genes are not considered to differ in terms of (i) *sequence* or (ii) level of *expression* (Wizemann, & Pardue, 2001). Firstly, gene *sequence* refers to the chemical composition of genetic material which is composed of a substance called deoxyribonucleic acid (DNA). DNA acts as a blueprint that codes for the synthesis of cellular proteins (required for the structure, function, and regulation of tissues) and is, in turn, composed of a linear sequence of nucleotides. These are repeating structural units of nucleic acids composed of sugar, phosphate and one nitrogen-containing base – adenine (A), thymine (T), guanine (G), or cytosine (C) – and it is the linear order of these bases along a DNA molecule that encodes the information necessary for protein synthesis (Brooker, 2005**;** Cummings, 2014). That is, proteins are constructed from amino acids and a three- base sequence (i.e., ATG) specifies one particular amino acid (of a possible 20).

An individual's DNA is contained within large structures known as chromosomes. The human genome consists of 46 chromosomes comprised of 22 pairs of autosomes (i.e., any non–sex-determining chromosome) and one pair of sex chromosomes (Jones & Lopez, 2014). Males and females have the same configuration of autosome. Therefore, genes located on these autosomes are similar for men and women and differences occur due to inheritance patterns and mechanisms of gene expression (Wijchers, & Festenstein, 2011). Further, the

genes considered to be influenced directly by an individual's genetic sex are located on the sex chromosomes (Wizemann, & Pardue, 2001).

Genes located on either of the sex chromosomes result in male- and female-specific gene expression and are therefore considered to contribute largely to sexual dimorphisms between men and women. Secondly, gene *expression* refers to the process by which gene products (cellular proteins) are synthesised in order to carry out cellar functions and thus influence an organism's traits (Brooker, 2005). The expression of genes located on the sex chromosomes varies between men and women due to differences in the dosage, or number, of genes. That is, particular genes are expressed differently between the sexes, with particular genes expressed at higher or lower levels in one sex versus the other. These genetic mechanisms and their effects will be explored in greater detail in section 2.2 (pages 27-38) below.

The process of sex determination begins with the allocation of genetic material via chromosomal complement and finishes once the development of sex-specific gonadal phenotypes (i.e., testes or ovaries) is initiated. This process is directed by the sex chromosomes (Ngun et al., 2011) as it is their specific combination that determines the individual's sex as either male or female. A "male" genotype is characterised by the presence of an X and a Y chromosome (XY) where the X chromosome is specifically inherited from the mother and the Y chromosome specifically from the father. In contrast, a "female" genotype is characterised by the presence of two X chromosomes (XX), one inherited from the mother and the other inherited from the father (Blakemore et al., 2013; Davies & Wilkinson, 2006; Neave, 2008). The sex chromosomes are believed to have evolved from a pair of autosomes and, through the progressive degradation of the Y chromosome, fewer genes on the Y chromosome have an active counterpart on the X chromosome (Mangs & Morris, 2007; Ross, 2005). There are, however, specific regions on the Y and X chromosomes which are homologous. These

shared regions, referred to as PAR1 and PAR2, are not sex-specific and therefore pair and recombine during meiosis similarly to an autosome (Mangs & Morris, 2007). The non-recombining regions of the X and Y chromosomes contain genes that do have homologs on the other chromosome and genes that are X or Y chromosome specific (Schaafsma & Pfaff, 2014). Genes located on either of the sex chromosomes are believed to influence sexual dimorphism either directly or by regulating the transcription of autosomal genes (Lenroot & Giedd, 2010). The expression of sex chromosome genes may be limited to sexually dimorphic tissues (e.g., breast tissue) or they may be expressed in both sexes but influenced through differential hormonal action brought about by male- and female-specific hormonal profiles (Wizemann, & Pardue, 2001). Arnold and Burgoyne (2004) however, suggest that each cell in an individual's body is characterised by the sex chromosome complement and thus XX cells and XY cells may be inherently different regardless of the hormonal environment.

The chromosomal profiles of humans can be further described with reference to a karyotype which is defined as the number, size, and shape of chromosomes in an organism. The sex chromosomes differ in size and genetic composition, with the X chromosome being the larger of the two and responsible for carrying many genes necessary for normal functioning (Arnold & Burgoyne, 2004; Blakemore et al., 2013). Despite the Y chromosome being smaller and encompassing fewer genes, it is considered to be the sex-determining chromosome as, simply stated, its presence or absence dictates male or female sex (Blakemore et al., 2013; Jones & Lopez, 2014). As demonstrated in Figure 2 (page 30) below, the karyotype of a typically developing human consists of the aforementioned 22 pairs of autosomes and 1 pair of sex chromosomes, where two X chromosomes paired together guide further biological (e.g., anatomical) development along a female trajectory, and the presence of an X and a Y chromosome pairing promotes further biological development along a male trajectory.

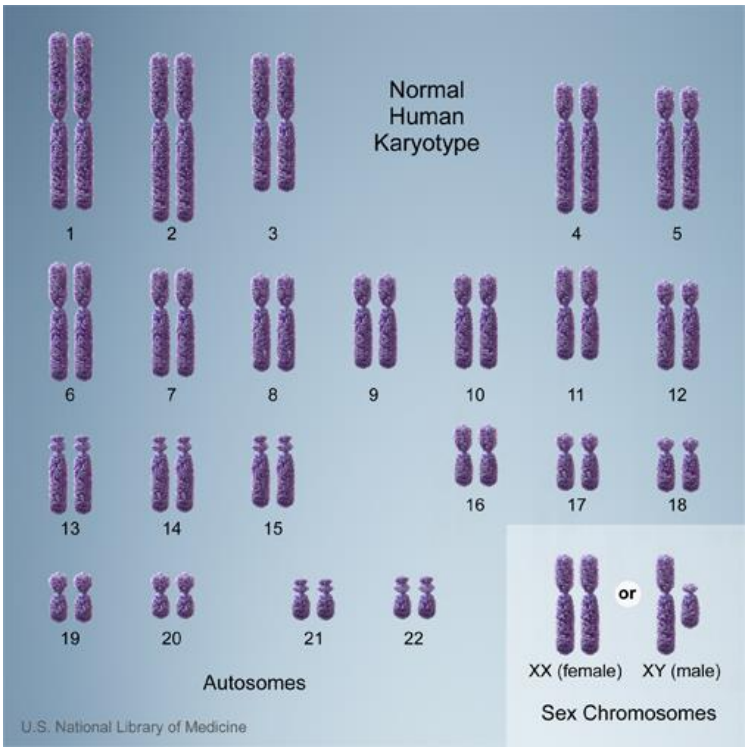

Figure 2: Depiction of a normal (typical) human karyotype. Sourced from 'Genetics Home Reference', by U.S. National Library of Medicine, 2015, http://ghr.nlm.nih.gov/handbook/illustrations/normalkaryotype

The Y chromosome contains a specific gene, called the sex-determining region of the Y chromosome, or *SRY* gene and it is the presence of this gene in the male karyotype that triggers the development of male-specific genitalia from undifferentiated anatomical structures, including the gonadal ridges, internal ducts and germ cells (Becker, Nylen, & Snider, 2001; Blakemore et al., 2013). In contrast, the absence of the *SRY* gene in the female karyotype guides the development of female-specific genitalia. These structures are referred to as 'bipotential' because they can assume a male or female form, this process is under the influence of steroid hormones in early development (see section 2.3 [pages 39-43] for detailed discussion of this process).

Males and females share similar autosomal genotypes in terms of the DNA sequence, gene structure and frequency of polymorphisms (i.e., the presence of two or more phenotypic

forms in a population). While naturally occurring variation within autosomal genes may contribute to different anatomical, physiological and behavioural traits, the vast phenotypic diversity that does exist between the sexes suggests that different regulatory pathways are responsible, at least in part, for sexually dimorphic traits linked to the autosomes (Ellegren & Parsch, 2007; Ober et al., 2008; Yang et al., 2006). This suggests that differences in autosomal gene expression, as opposed to the genes themselves, may be responsible for the phenotypic differences observed between men and women. Yang et al. (2006) for example, conducted a large-scale microarray analysis on 334 mice and identified wide spread sexually dimorphic gene expression between males and females in all four of the somatic tissues analysed, including the brain, liver, adipose tissue, and muscle. Yang et al. (2006) reported the degree of sexual dimorphism ranged from ~14% in the brain to ~70% in the liver. In addition, these sexually dimorphic genes showed evidence of exhibiting tissue-specific patterns of expression (i.e., genes predominantly expressed in a particular tissue) along with chromosomal enrichment on both the sex chromosomes and autosomes. It should be noted however, that such sex differences are also directly and indirectly mediated by the effects of gonadal hormones. Still, as genetic differences between males and females occur in the sex chromosome complement, a number of sex differences can be attributed to the sex chromosomes (Davies & Wilkinson, 2006; Ellegren & Parsch, 2007).

Genes positioned at particular physical locations (called “loci”) on homologous chromosomes (two chromosomes, one of paternal origin and one inherited from the mother that are similar in appearance and synapse during meiosis, Hyde, 2009) are present in pairs. A gene pair that consists of two alternative forms of a gene are referred to ‘alleles’ and contribute to differing male/female phenotypic expression depending on the specific allele’s recessive or dominant nature. Dominant alleles determine the phenotype in a heterozygous

condition (having two different alleles of the same gene), whereas recessive alleles are masked by a dominant allele (Hyde, 2009).

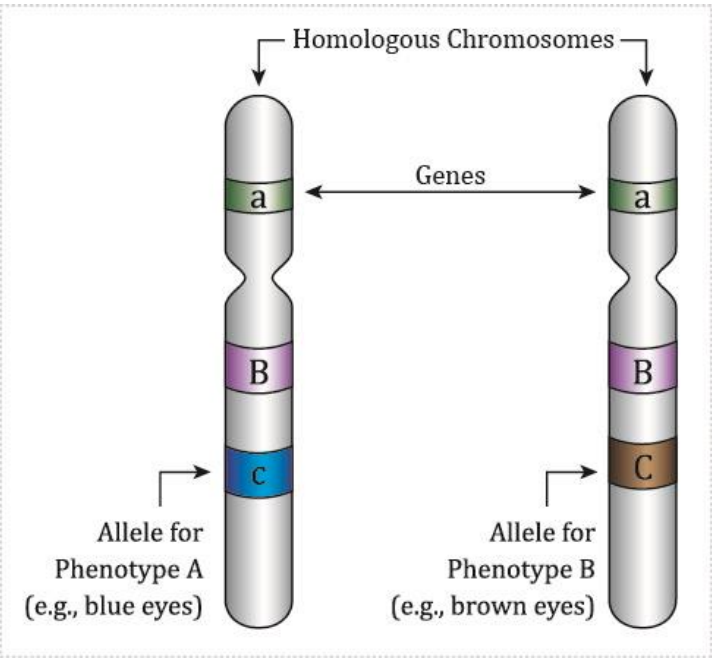

Figure 3: Depiction of homologous chromosomes with three difference genes. Different forms of the same gene are referred to as alleles. These two chromosomes are homozygous for the recessive allele of gene 'A' and the dominant allele of gene 'B'. These chromosomes are heterozygous (Bb) for gene 'B', whereby the 'B' allele is dominant over the recessive 'b' allele. Adapted from Brooker, R. J. (2005). Genetics: Analysis and principles (2nd E.d.). New York, NY: McGraw-Hill.

Genes at particular loci on the sex chromosomes are described as being sex-linked, meaning that they are found only on either of the X or Y chromosomes (Brooker, 2005). In females, with an XX chromosome combination, a recessive allele (one that produces its characteristic phenotype only when paired with an identical allele) on one X chromosome is often masked if a dominant allele (one that produces a characteristic phenotype whether the paired allele is identical or different) occurs on the other X chromosome (Brooker, 2005). In contrast, males possess only one X chromosome and this limits the gene sequence at a

particular locus to one. As the X chromosome contains significantly more genes than does the Y chromosome, only a small portion of the Y chromosome has a homologous (i.e., the same genes at the same loci) complement on the X chromosome; therefore, X-linked recessive genes on the X chromosome are expressed in the male phenotype (Wizemann, & Pardue, 2001). Similarly, genes carried on the Y chromosome can only be expressed in males (Blakemore et al., 2013; Davies & Wilkinson, 2006; Jones & Lopez, 2014). Y chromosome genes have been implicated in the development and growth processes that give males some of their sexually dimorphic traits.

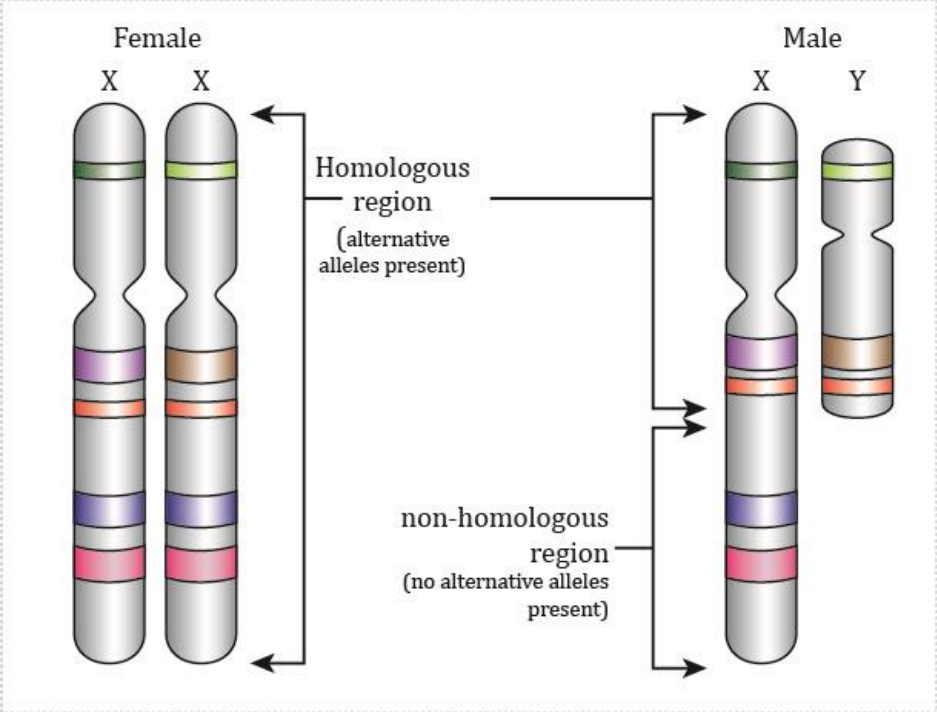

Figure 4: Comparison of the homologous and non-homologous regions of the X and Y chromosomes. In the female (XX) complement alternative alleles are present for all genes. In the male (XY) complement however, alternative forms are only present in the homologous regions. Adapted from Brooker, R. J. (2005). Genetics: Analysis and principles (2nd E.d.). New York, NY: McGraw-Hill.

Male-female differences in sex-linked gene expression also arise due to two processes which influence inheritance patterns: *dosage compensation* and *genomic imprinting*. Dosage compensation is the equalisation of gene expression between males and females and works to

offset the difference in the number of active sex chromosomes between the two sexes. This process is necessitated by the loss of recombination between X and Y chromosomes. As the Y chromosome evolved and Y-linked genes degenerated or changed function, some genes on the male X chromosome were left without a homologous equivalent on the Y chromosome, requiring either the up-regulation of male X-linked genes (as they are present in half the dose than they are in females) or the down-regulation of X-linked genes in the female karyotype (Esser, Sultera, & Fein, 2010**;** Schaafsma & Pfaff, 2014). Dosage compensation creates equivalence in the phenotypic expression of characteristics determined by genes on the X chromosome (Blakemore et al., 2013; Lenroot & Giedd, 2010; Yankelevitch-Yahav & Yankelevitch-Yahav, 2014). Specifically, because the female karyotype is characterised by the presence of two X chromosomes, females inherit double the number of alleles necessary to code for X-linked characteristics. As both alleles do not function in the same cell, equalisation is achieved in females by one of the two X chromosomes being randomly turned off in a process referred to X-inactivation, which results in half the number of inherited genes being expressed (Blakemore et al., 2013; Lenroot & Giedd, 2010). Yet, in order to equalise gene product between males and females, some genes may escape inactivation (approximately 15%-25%), thus resulting in a higher expression of these genes in females than in males and contributing to phenotypic sex differences between the sexes (Berletch, Yang, Xu, Carrel, & Disteche, 2011; Richardson, 2010). Interestingly, a female X chromosome can be active in one cell and inactive in another, and either of the parental X chromosomes (the one inherited from the mother or the one inherited from the father) can be functional. This suggests that factors relating to gene regulation (i.e., epigenetic factors) are implicated in x-inactivation patterns, and may also contribute to sex-differences between males and females in health and disease (Migeon, 2007; Ober et al., 2008).

Genomic imprinting produces a change in a single gene or chromosome during gamete formation and concerns the exclusive expression of inherited genes from the parent (Schanen, 2006). During spermatogenesis (process of sperm cell development) and oogenesis (process of ova development) these alterations in genes are marked, or imprinted, on the parental chromosome. An imprinted gene is haploid, meaning that only one allele works while the other is silenced. This results in the expression of only one copy of the gene despite two copies being inherited from the parents. In addition, recessive genes that would not normally be expressed may be expressed if the gene is imprinted and the dominant complement is silenced (Brooker, 2005**;** Jirtle & Weidman, 2007). Through the processes of dosage compensation and genomic imprinting, sex-linked inheritance generates sex-specific differences in the expression of genes on the X and Y chromosomes and acts independently of, and prior, to the introduction of other factors, such as hormonal influences (Craig, Harper, & Loat, 2004). Thus, due to the different complement of genes, each cell in the human body is characteristically male or female and these sex-specific genetic differences contribute to subsequent phenotypic differences between the two sexes.

Mechanisms of inheritance generate genetic variability between men and women, by determining which genes are present in the individual's genotype, depending on the manner in which genes are expressed, although it is important to note that these mechanisms contribute to variability within the sexes as much as they do between the sexes (Craig et al., 2004; Ngun et al., 2011; Rutter et al., 2003). There are a number of mechanisms that impact genetic expression among individuals (such as the chemical modification of genes and the presence of regulatory proteins to turn them on or off) many of which are influenced by the environment. However, it may be that sex-specific regulatory networks exist within the sexes and that these interact with male and female genotypes, thus contributing to sex-specific differences in phenotypes (Ober et al., 2008). Such interactions would account for phenotypic

variation between the sexes without negating variation within the sexes. Evidence of direct sex chromosome effects in sexual dimorphisms initially came from research, which demonstrated size differences between XY and XX pre-gonadal embryos. Scott and Holson (1977), for example, found that 12-day old male rat embryos weighed more and possessed more protein than did female rat embryos of the same age and that male embryonic growth occurred at a faster rate than that of the female embryo. These differences occurred prior to the sexual differentiation of the male-specific and female-specific gonads and thus in the absence of a male- or female-specific hormonal profile, suggesting that some differences (in embryonic development) that exist between the sexes may be attributed to the sex chromosomes. Burgoyne et al. (1995) have proposed that such findings are due to complex genetic effects involving both the X and Y chromosomes. More specifically, those researchers identified that the Y chromosome was responsible for accelerated growth in XY mice embryos as opposed to XX mice embryos aged 10.5 *days post coitum* and that this difference was not specifically linked to the *SRY* gene, implicating other Y chromosome genes as influential in this sex difference. In addition, Burgoyne et al. (1995) suggested that differences in X chromosome constitution were also responsible for the sex-based variation observed between XY and XX mice embryos. That is, difference in the activity of the X chromosomes contributes to XX-XY sex differences and may be attributed to variation in sex-linked gene expression. Studies such as these suggest that variant gene compliments between XX and XY genotypes are indicative of intrinsic sex differences that are present in an embryo prior to the differentiation of physiological and anatomical characteristics in early development and thus prior to the influence of gonadal hormones (Arnold & Burgoyne, 2004; Maatouk & Capel, 2010).

Additional evidence for sex chromosome effects on sex differences has been generated via mouse models designed to separate sex the chromosome complement from gonadal phenotype (Wijchers, & Festenstein, 2011). One such model, referred to as the Four-Core-

Genotypes model, allows for comparison of the relative contribution of sex chromosomes and hormones as well as the interaction between these two factors (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012; Pankevich et al., 2011). This model has been applied in genetically-modified mice in which the *SRY* gene is deleted from the Y chromosome and transgenically inserted into an autosome. This modification process results in four possible genotypes: XX and XY mice without the *SRY* gene, which develop ovaries, and XX and XY mice with the *SRY* gene, which develop testes (Lenroot & Giedd, 2010). Subsequent comparisons of these genotypes distinguishes sex differences directed by X or Y genes and those directed by hormones (Pankevich et al., 2011).

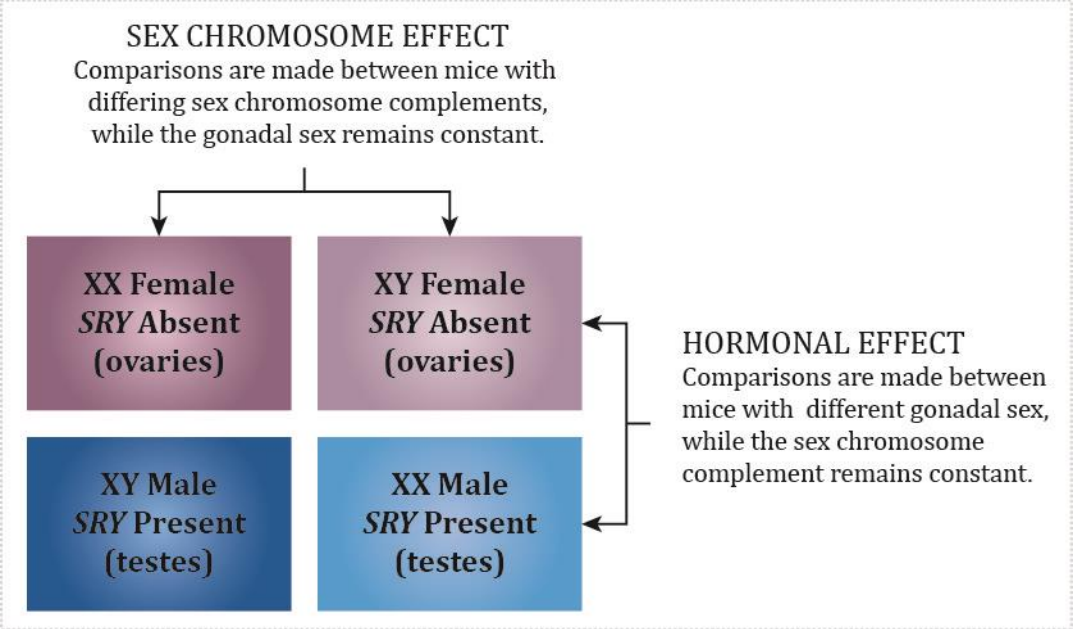

Figure 5: The FCG model allows a 2 x 2 comparison of four possible genotypes to detect the phenotypic effects of hormones (gonadal type) and sex chromosome (XX vs. XY). Adapted from Arnold, A. P and Chen, X. (2009). What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? Frontiers in Neuroendocrinology, 30(1), 1-9. doi:10.1016/j.yfrne.2008.11.001.

While this model has identified the influence of hormonal factors in the development of sex differences in anatomical structure and behaviour, it has also revealed that dosages of

sex chromosome genes also play a role (McCarthy et al., 2012**;** Quinn, Hitchcott, Umeda, Arnold, & Taylor, 2007). Using this model, Quinn, Hitchcott, Umeda, Arnold and Taylor (2007) for example, found sex differences in the rate of habit formation where XX mice showed faster food-reinforced habit formation over XY mice, regardless of gonadal phenotypes, suggesting a direct influence of sex chromosomes genes in this behaviour. In contrast to sexual determination which is focused on genetic inheritance and expression, sexual differentiation is biochemical in nature and drives the impacts of various hormones on the development of sexually-dimorphic characteristics. In examining sex differences in humans, the relative influence of both sex chromosome complement and hormones requires further examination to understand the development of sexually-dimorphic structures (such as the brain) and the subsequent influences that differentiated structures have on behaviour (such as aggression).

2.3: The Influence of Hormones on Human Sexual Dimorphism

The discussion in this section of Chapter 2 aims to explain the second mechanism of male—female differentiation, which is essentially of a biochemical nature. The process of sexual differentiation, which follows genetic sex determination, involves the development of the male- and female- specific anatomical phenotypes, including sexual anatomy and brain characteristics and occurs under the influence of hormones. Hormones are molecules produced and secreted by the gonads and other glands in the human body and act to create specific responses in specific cells (Neave, 2008). Hormones control physiological functions and coordinate processes such as growth, metabolism, and reproduction by acting as chemical messengers, exerting their influence through interactions with high-affinity receptors to bring about a biological response (Gardner, Anderson, & Nissenson, 2011). Hormones are classified according to their chemical composition. Hormones belonging to specific chemical groups, whilst being structurally-different, exhibit similar properties and functions due to their chemical uniformity. The major hormone categories are represented as amino-acid derivatives, peptides or polypeptides, fatty acid derivatives, and steroids (Becker et al., 2001). Steroids, produced in early embryonic development and across the lifespan, contribute significantly to morphological, physiological, and behavioural sexual dimorphisms between the sexes and will be the focus of the subsequent discussions presented in Chapter 3 (pages 44-63) and Chapter 4 (pages 64-98).

The steroid group of hormones, also referred to as sex hormones or sex steroid hormones, are produced by the gonads and adrenal glands and include androgens, oestrogens, progesterone, glucocorticoids, and mineralocorticoids. These hormones control the differentiation and growth of the reproductive system, induce and maintain sexual characteristics and modulate reproductive behaviour (Giguère, Yang, Segui, & Evans, 1988). Steroid hormones also have important implications for gene regulation. Steroids enter target

cells by diffusing through the extracellular space and binding with complex nuclear receptors located within the cytoplasm cell (Burns et al., 2003). Once bound, the steroid/receptor complex enters the cell nucleus and undergoes a change to allow it to bind to a regulatory region of a steroid-responsive gene, thereby regulating gene expression by turning on and off transcription of that gene (Jones & Lopez, 2014; Parker, 1988). The expression of genes is likely to be influenced by the differing hormonal profiles that exist between the sexes. Whereas some genes may only be expressed in sexually dimorphic tissues, others may be expressed globally but could possibly be subject to hormonal regulation in different tissues throughout development (Wizemann, & Pardue, 2001).

Prior to sexual differentiation there are two sets of genital ducts present and the process of differentiation causes one of these to develop further and the other to disappear. The SRY gene codes for the transcription factor protein called testis-determining factor, which initiates the development of testis around weeks 6-7 of gestation (Hines, 2010; Wizemann, & Pardue, 2001). In males, testes then produce male sex hormones, called androgens, which further the process of differentiation by promoting the development of masculine features (Pankevich et al., 2011). Specifically, Wolffian ducts develop under the influence of high levels of androgens produced by the Leydig cells, which further differentiate to form the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts of the male internal genital system (Neave, 2008; Wizemann, & Pardue, 2001). Simultaneously, Müllerian inhibiting substance (MIS) is produced and acts to cause the Müllerian ducts (the female equivalent of the Wolffian ducts) to regress (Jones & Lopez, 2014). The final stage of differentiation, the development of external genitalia, occurs under the influence of dihydrotestosterone (DHT). Produced directly from testosterone, high levels of DHT promote the development of male genitalia, including the penis and scrotum. In contrast, in females, due to the absence of the *SRY* gene in the female karyotype, the undifferentiated gonads develop into ovaries, which these have no

effects on the development of female genitalia. Instead it is the subsequent lack of androgens (specifically MIS) which causes the Wolffian ducts to degenerate and the Müllerian ducts to develop into the uterus, fallopian tubes, and upper parts of the vagina (Neave, 2008).

The normal development of male and female sexual characteristics has important implications for a typically developing individual. Firstly, the gonads, in conjunction with other endocrine glands, continue to influence anatomical, physiological and behavioural development across the lifespan through hormone production and action (Nugent et al., 2012). The development of secondary sex characteristics (also under the influence of gonadal hormones) during puberty as well as emotional and cognitive advancement in adolescence are examples of such influences. Second, the presence of male- or female-specific external genitalia may influence socio-cultural expectations imposed upon the individual (Fagot, 1978). For example, parents, who act as major socialisation agents for children in regards to their children's play behaviour, are more likely to reinforce children more positively when they are engaged in play activities considered to be gender appropriate (Berenbaum, Martin, Hanish, Briggs, & Fabes, 2008). Socio-cultural factors however, are explored further in Chapter 4 (pages 64-98) and will not remain a focus of this discussion. Traditional views of sexual differentiation considered the gonadal hormones to be almost exclusively responsible for the sexual dimorphism of male and female characteristics such as sexual anatomy, muscle mass, bone structure and, most importantly, the brain. While it is known that hormonal factors interact with other factors such as genetics, the subsequent discussion focusses on the unique influence of gonadal hormones on brain development, as these hormonal influences have ongoing influence on brain function and subsequent behaviour (Davies & Wilkinson, 2006; Maatouk & Capel, 2010; Ngun et al., 2011).

One important role of the gonadal hormones is to direct brain development along a male- or female-typical trajectory by exerting two major influences on the developing brain:

organisational effects and activational effects. These effects act to masculinise or feminise the brain and neural circuitry, subsequently influencing behaviour. Hines (2002) describes masculinisation as the "movement along a continuum from none of a characteristic that is more common in males than in females to the greatest possible amount of the characteristic" (pg. 426). Exposure to male sex hormones promotes this development and influences male-typical behaviours in the adult male (Sato et al., 2004). In contrast, feminisation refers to those continuum-based characteristics that are more typical of females than males (Hines, 2002) and the lack of androgens in early development influences female-typical behaviour in the adult female.

O*rganisational effects* occur during specified critical periods in brain development and produce permanent anatomical and/or physiological outcomes by directing the formation of neurological pathways that underlie behavioural responses (Berenbaum, Blakemore, & Beltz, 2011). These effects are considered to be asymmetric because androgens are required for brain masculinisation but oestrogens are not required for brain feminisation (Neave, 2008). Neufang et al. (2009) demonstrated the organisational effects of hormones on the brain in a study which revealed that the amount of grey matter in the amygdala was predicted by testosterone levels in adolescent males and females.

Activational effects are considered to be temporary and do not exert long-term developmental outcomes or result in permanent structural changes (Berenbaum et al., 2011; Blakemore et al., 2013). Instead, these effects are often linked to changes in adolescent and adult behaviour where a specific behaviour is only displayed as long as the hormone is present. These transient effects do not have any developmental restrictions but can be constrained by organisational effects. That is, activational effects can only occur if the associated neural circuits have already been organised (Bao & Swaab, 2010). Finally, activational effects are considered to be symmetrical because androgens are required to

induce male-typical behaviours and oestrogens are required to induce female-typical behaviours (Neave, 2008). An example of the activational effects of hormones is the cyclical rise and fall in levels of ovarian hormones throughout the female oestrous cycle which influences many behaviours including cognition (Hampson, 1990; Maki et al., 2002). Although gonadal hormones are commonly considered for their effects on sexual reproductive behaviours, they are also known to be involved in sexually dimorphic behaviours including emotion and cognition (Barth, Villringer, & Sacher, 2015; Giedd, Raznahan, Mills, & Lenroot, 2012).

The review of studies presented in this section of the thesis supports the conclusion that both genetic and biochemical (hormonal) factors contribute to sexually dimorphic characteristics and may lead to differences in the way men and women interact with their environments. The sex chromosomes may have mediating effects on sex differences, directly and independently of sex hormones (Davies & Wilkinson, 2006; Lenroot & Giedd, 2010), as evidenced by the presence of identifiable differences before the introduction of sex hormones at approximately six weeks of gestation (Burgoyne, Thornhill, Boudrean, Darling, Bishop, & Evans, 1995; Craig et al., 2004; Davies & Wilkinson, 2006). The effects of steroid hormones can be observed across the lifespan. Prenatally, they promote differentiation in the development of male and female brains and bodily characteristics and influence brain physiology and behaviour throughout the lifespan (Yankelevitch-Yahav & Yankelevitch-Yahav, 2014).

Chapter Three: Sex Differences in the Brain

Of particular interest in the examination of sex- and gender-based differences between men and women is the investigation of variation in the structural and functional features of the brain and how that variation may influence sex-based differences in cognition and behaviour (Cosgrove et al., 2007; Hines, 2010). Brain structure is influenced by environmental, developmental, and genetic factors (Cosgrove et al., 2007; Yankelevitch-Yahav & Yankelevitch-Yahav, 2014) and complex interactions between these factors result in highly variable brain features within sexes and across the sexes (Yankelevitch-Yahav & Yankelevitch-Yahav, 2014). Genes are involved in determining the formation of neural circuits in the developing brain, which are further influenced by hormones present during early development and across the lifespan (Craig et al., 2004). Sex differences in gene expression may emerge in the form of different response patterns to certain tasks and the ongoing interactions an individual has with his or her environment (Cosgrove et al., 2007; Hines, 2010; Lenroot & Giedd, 2010).

The extent to which structural differences in the brain exist is currently under debate in the research. Despite that debate, there is a strong line of research which suggests that structural variation in the brains of males and females might contribute to observable sex differences in behaviour patterns such as those involved in socialisation, physical aggression and in specific cognitive abilities (e.g., emotion, memory, perception and language) (Crick & Zahn-Waxler, 2003; Gong, He, & Evans, 2011). The discussion in Sections 3.1 (pages 45-52) and 3.2 (pages 53-63) below presents a summary of the key research findings on male-female brain differences and the implications of those differences on behaviour and interactions in the social environment.

3.1: Development of the Sexually Dimorphic Brain

Brain development is largely directed by the actions of steroid hormones following the development of male or female internal genitalia, although many of these actions are linked to the genome as they occur at the level of gene transcription through steroid hormone-DNA-binding receptor interaction (Hadley, 2000; Parker, 1988). The key hormonal factor in brain differentiation is testosterone: its presence in the male foetus induces the masculinisation of the brain and its absence in the female foetus promotes the feminisation of the brain (Gillies & McArthur, 2010). Once testosterone has entered the brain of the male foetus it is converted into (i) 5α-DHT through its interaction with 5-alpha-reductase enzyme (which reduces testosterone to DHT) and (ii) estradiol by the catalysing enzyme aromatase (a process referred to as the aromatisation of testosterone or the aromatisation hypothesis) (Roseli, Liu, & Hurn, 2009). Estradiol acts on estrogen receptors and influences developmental processes such as neurogenesis (the formation of neurons, Purves et al., 2012), apoptosis (cell death due to a programmed pattern of gene expression, Purves et al., 2012), and migration (the positioning of cells into appropriate spatial relationships, Purves et al., 2012) resulting in organised regional sex differences in the number of cells and their distribution within specific regions (Davis, Popper, & Gorski, 1996; Gillies & McArthur, 2010; Tobet et al., 2009). There is also emerging evidence to suggest that androgens acting on androgen receptors within the brain also influence the development of the masculine brain (Zuloaga, Puts, Jordan, & Breedlove, 2008). Both testosterone and estrogen have major effects on neuronal growth and development, indicating that sexual dimorphism within the brain is mediated by the hormonal environment (i.e., the epigenetic action of steroid hormones) (Kawata, 1995). Low levels of estrogens are required for brain feminisation, whereas high levels of estrogens generated in the male brain result in brain masculinisation and it is the regulation of estrogen that promotes the development of the sexually dimorphic brain (Kawata, 1995).

One well recognised and highly researched structure of the brain is the sexually dimorphic nucleus (SDN), a large cluster of neuronal cells located in the preoptic area (POA) of the hypothalamus. This region has been implicated in the control of male- and female-specific patterns of sexual behaviour and endocrinology in adults (Arai, Sekine, & Murakami, 1996; He, Ferguson, Cui, Greenfield, & Paule, 2013; Sakuma, 2009). The critical period for the development of this region begins around embryonic day 10 and terminates at postnatal day 10 (Wada-Kiyama et al, 2013). The SDN-POA is testament to the organisational and activational effects of hormones because during development, this region expresses gonadal hormone receptors and aromatase and, once established, the neural circuits correlate strongly with the expression of sexual behaviour (Wright, Schwarz, Dean, & McCarthy, 2010). Morphologically, (largely due to the presence of a greater number of cells), the SDN-POA of male rats is typically 3-8 times larger than that of female rats. Postnatally, the male SDN-POA expands continuously through to adulthood while in females it remains at relatively the same size (He, Ferguson, Cui, Greenfield, & Paule, 2013**; 230**). Such differences have been attributed to the aromatisation of testosterone to estradiol during development (Davis et al., 1996; Gorski, Harlan, Jacobson, Shryne, & Southam, 1980) because the administration of gonadal steroids to castrated male rats does not alter the volume of the SDN-POA (Gorski, Gordon, Shryne, & Southam, 1978). There are also specific periods that impact the development of the SDN. Dohler et al. (1984) demonstrated that postnatally administering testosterone propionate or diethylstilbestrol (a synthetic estrogen) to female rats causes the SDN-POA to permanently develop to the same size as that seen in males, where this hormone treatment is started prenatally (see Figure 6 [page 47] for a comparative diagram of the results of this study). Further, the administration of testosterone to neonatal (the developmental period from birth to 1 month) female rats also resulted in an increase in the volume of the SDN-POA in adulthood compared to control females, but not to a size of that normally seen in males,

while the castration of neonatal males reduced the volume of this region in adulthood (Gorski et al., 1978). These findings suggest that the development of the sexually dimorphic nucleus occurs in a step-wise or sequential manner (Kawata, 1995) and that differing hormonal profiles between men and women contribute to the development of sexually dimorphic brains.

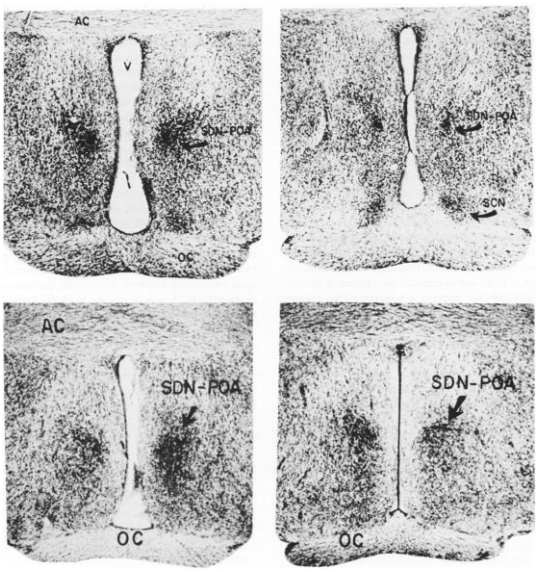

Figure 6: Coronal sections of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in adult rats showing: a male (top left) and a female (top right) rat treated pre- and postnatally with oil (placebo) and female rats treated pre- and postnatally with testosterone propionate (bottom left) or with diethylstilbestrol (bottom right). Sourced from Dohler, K. D., Coquelin, A., Davis, F., Hines, M., Shryne, J. E. and Gorski, R. A. (1984) Pre- and postnatal influence of testosterone propionate and diethylstilbestrol on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. Brain Research, 302, 291-295. doi:10.1016/0006-8993(84)90242-7

The influence of testosterone metabolites is also evident in the development of the neuronal networks that exist within the POA. The primary function of the nervous system is signalling, or information transfer, and this process occurs through specialised cells called neurons (Levitan & Kaczmarek, 2002). In turn, the neuron is comprised of three major regions responsible for facilitating the transfer of information: the cell body, a single axon, and

dendrites. The *cell body* contains the nucleus, associated cytoplasm, and other cell structures required for functioning. The *axon* extends from the cell body and is responsible for transmitting electrical signals from the cell body. When a neuron is active, an electrical current (referred to as an action potential) is transmitted along the axon to the synaptic terminal. Also protruding from the cell body and extending over grey matter are a varying number of *dendrites*, or *dendritic shafts* and from the dendric shaft are small projections called *dendritic spines*. These are the synaptic input sites at which the neuron receives information from other cells and are therefore sensitive to electrical and chemical activity (Hof et al., 2014; Levitan & Kaczmarek, 2002; Schwarz & McCarthy, 2008). Independent neurons are connected by the *synapse*, which can be defined as the functional and structural site at which transmissions between cells occur. There are two classes of synapse: electrical and chemical (see Figure 7 [page 49] for a depiction of the differences between electrical and chemical synapses). In an electrical synapse the cell membranes of two communicating neurons are connected by channels (termed gap junctions or connexons), which are capable of passing an electric current (Levitan & Kaczmarek, 2002; Purves et al., 2012). Chemical synapses are characterized by a small gap, termed the synaptic cleft, which separates two cells. When an action potential reaches the axon terminal, chemicals called neurotransmitters are released into the synaptic cleft where they bind to receptors located on the adjacent neuron dendrites to create a synaptic potential. When this synaptic potential reaches the axon of the receiving neuron it triggers an action potential in this neuron that is then sent along the axon.

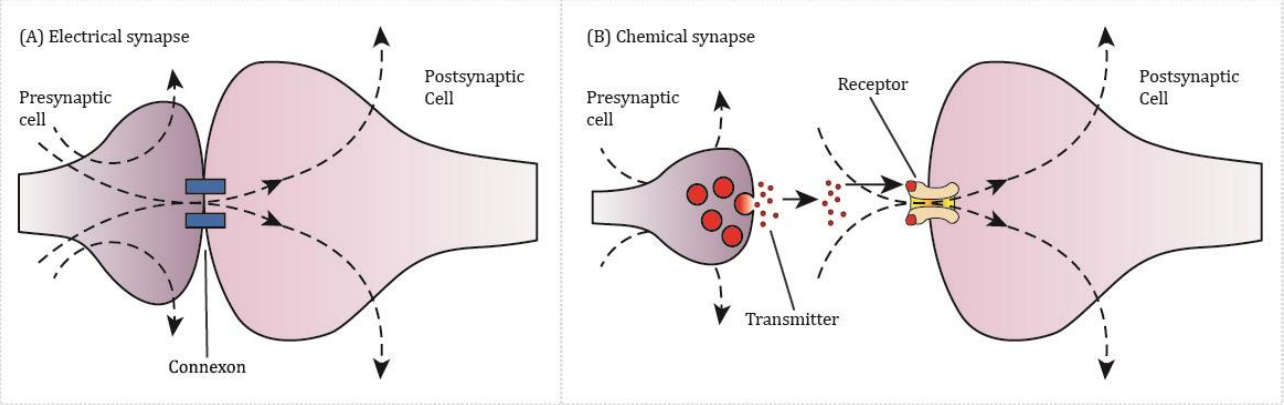

Figure 7: (A) At an electrical synapse, electrical currents pass directly from one cell to another through connexions. (B) At a chemical synapse, the release of neurotransmitter molecules from the presynaptic cell opens receptors on the postsynaptic membrane, causing excitation of inhibition. Adapted from "Mechanisms of Direct Synaptic Transmission" by J. G. Nicholls, A. R. Martin, P. A. Fuchs, D. A. Brown, M. E. Diamond, and D. A. Weisblat, 2012, *From Neuron to Brain*, p. 186. Copyright 2012 by Sinauer Associates, Inc.

It is through the chemical (the manner in which steroid hormones exert their effects through gene regulation) and distributional (diffusion via extracellular space) properties of steroid hormones that brain development, metabolism and function are mediated (Kawata, 1995). Nerve cell differentiation and the subsequent structure of neuronal networks within the brain can be regulated by steroid hormone action. This in turn modulates neural communication thereby controlling behaviour such as sexually dimorphic responses and stress-responsive adaptations (Kawata, 1995; Roseli et al., 2009).

Early developmental exposure to estradiol results in permanent effects on the development of sexually dimorphic synaptic patterning within the POA. Dendritic spine synapses occur at greater density and there also exist more complex astrocytes (a type of glial cell found only in the central nervous system that acts to maintain an appropriate chemical environment for neuronal signaling, Purves et al., 2012) in the male POA compared to that of the female (Schwarz & McCarthy, 2008; Wright et al., 2010). The mechanism by which these

sex differences arise under the influence of estradiol was discovered by Amateau and McCarthy (2002a) when they found that administering prostaglandin E_2 (PGE_2) to neonatal female rats increased the number and density of dendritic spines in the POA. Briefly, prostaglandins are biologically active molecules that maintain homeostatic functions and mediate pathogenic mechanisms, including pain and inflammatory responses (Ricciotti & FitzGerald, 2011). Their synthesis is dependent on the activity of prostaglandin G/H synthases, COX-1 and COX-2, the isoforms of cyclooxygenase (Ricciotti & FitzGerald, 2011). During development COX-2 is higher in the male POA compared to females; treating females with estradiol increases COX-2 in this region, which then up-regulates the production of prostaglandins, including PGE_2, resulting in the aforementioned increase in the number and density of dendric spines in the region. In a similar study Amateau and McCarthy (2002b) also demonstrated that the administration of estradiol influences the morphology of astrocytes within the POA. The treatment of newborn female pups with estradiol resulted in a significant increase in the number of primary processes per astrocyte suggesting that gonadal steroids modulate astrocyte morphology resulting in the establishment of sexually dimorphic synaptic patterns.

Researchers (e.g., Forger, 2006 and Orikasa, Kondo, Usui, & Sakuma, 2010) investigating the developmental mechanisms by which this sexual dimorphism occurs suggest that the processes of neurogenesis, migration and apoptosis, all play some part in the creation of the sexually dimorphic POA, although the extent to which cell proliferation and migration are involved is debated (Forger, 2006; Orikasa, Kondo, Usui, & Sakuma, 2010). Orikasa, Kondo, Usui, and Sakuma (2010) found no sex differences in the number of neurons in the SDN-POA between males and females, despite notable sex differences in the volume of the nucleus. Sex differences were instead attributed to male-specific postnatal radial spreading of cells as opposed to embryonic neurogenesis. In contrast, Sakuma (2009) suggests that

mechanisms of cell proliferation and migration are regulated in site- and time-specific manners, and that they contribute, at least in part to sexual dimorphism in the POA. The role of cell death in the development of the SDN-POA, however, has been well established. Arai, Sekine, and Murakami (1996) injected female rats with estradiol benzoate (EB) to study the effect of estrogen on the occurrence of apoptosis in the SDN-POA. With the administration of estrogen, apoptosis was inhibited in the developing SDN-POA, leading the researchers to reiterate the importance of the aromatisation of androgen to estrogen in inducing sexual dimorphism in the SDN-POA. In another study that compared male and female SDN-POA development, Davis, Popper, and Gorski (1996) found a greater incidence of apoptosis in females between P7 and P10, which they suggested influenced an overall increased apoptosis in the first 13 days after birth, indicating that higher rates of cell death may result in fewer cells and thus a smaller nucleus in adult females. In that study, testosterone also appeared to reduce the incidence of apoptosis as shown by the administration of exogenous testosterone propionate to castrated males, which reduced the rate of apoptosis to a level below that of control males. Davis et al. (1996) suggested that this reinforced the role of gonadal steroids in the regulation of this process in developing animals.

Although the mechanisms that underlie the development of the SDN-POA are debated, this region demonstrates the potential influence of differing hormonal profiles in the development of the sexually dimorphic brain. Further research is required to better understand the manner in which various genetic and hormonal factors impact such processes and how these factors may subsequently contribute to the development of sexual dimorphism in the male and female brain. Of particular importance in such explorations is the extent to which morphological and physiological variations translate to behavioural differences. A large body of research has focused on male and female sexual behaviours in rodents and other animals (Cohen-Bendahana, van de Beek, & Berenbaum, 2005**;** McPherson & Chenoweth,

2012; Wallen, 2005) and how these behaviours are mediated by brain and hormonal differences between the sexes. Emerging research, in part due to technological advances in brain imaging techniques, is beginning to explore sex-based brain differences in humans and the impact of these on cognitive performance, personality traits, and emotional functioning.

3.2: Sex-Based Differences in Brain Morphology and Physiology

3.2.1: Techniques and mechanisms used to study brain morphology and physiology.

Investigations of sex-based variation in brain morphology and physiology have employed various methods, referred to as neuroimaging or brain imaging, to view activity or problems within the brain. The following discussion explores such techniques because subsequent sections (Section 3.2.2 [pages 57-63]) and Chapters (Chapter4 [pages 64-98], Chapter 5 [pages 99-121], and Chapter 6 [pages 122-156]) of this thesis refer to research findings from studies that have used neutoimaging techniques to explore sex differences in brain structure and function in men versus women. Neuroimaging techniques have significant utility in medical and psychiatric research and contribute to clinical care by providing the basis on which to explore brain morphology (structure), physiology (function) and brain chemistry (hormones) and the interactions between these factors (Cosgrove et al., 2007; Whittle et al., 2011). Many of these techniques are non-invasive and allow for the direct and quantitative measurement of the brain (Asbury, 2011). Neuroimaging techniques can be divided into those that examine brain structure (structural imaging), those that assess brain function (functional imaging) and those that examine brain chemistry (molecular imaging) (Asbury, 2011; Malhi & Dewan, 2001).

Structural techniques allow clinicians to view anatomical components of the brain such as white matter (WM) and grey matter (GM) as well as the movement of brain fluids across brain regions (Gong et al., 2011). In pathology, such techniques have particular utility in attempting to link behavioural deficits to specific brain regions (Petersen, Fiez, & Corebetta, 1992). Commonly used structural imaging techniques include computed tomography (CT) and magnetic resonance imaging (MRI). CT combines x-ray images into two-dimensional cross-sectional images of the brain and measures brain tissue density (Cosgrove et al., 2007;

Getz, 2014). MRI involves applying a strong magnetic field across the part of the body being assessed (in this case the brain) and provides an image intensity contrast between brain tissue types by measuring signals emitted from protons that exist in differing concentrations in those tissues (Purves et al., 2012; Ward, 2015). Brain function can be evaluated by measuring changes in regional brain activity across different brain states, which can be induced by the application and variation of cognitive or physical tasks or by administering drugs designed to alter brain states (Cosgrove et al., 2007).

Functional techniques measure brain activity and have contributed to the understanding of the mechanisms involved in cognitive, affective and motoric processes (Ray & Oathes, 2003). Functional techniques include functional magnetic resonance imaging (fMRI), voxel-based morphometry (VBM), and diffusion tensor imaging (DTI). fMRI captures neural activity by measuring blood flow to a particular region of the brain, under the premise that increased blood flow to an area reflects mental activation in that area (Dimoka, 2012). MRI measurement of changes in the level of oxygen in brain tissue is used to map neuroanatomical activation in response to cognitive tasks (Getz, 2014) and specifically targets the amount of deoxyhemoglobin in the blood. As neurons consume oxygen they convert oxyhemoglobin to deoxyhemoglobin, which produces a distortion in the local magnetic field. This distortion is the component that is measured to give an indication of the amount of deoxyhemoglobin in the blood (Ward, 2015). This technique has been termed BOLD (blood-oxygen-level-dependent contrast); as neural activity increases, the BOLD signal changes in response (Ward, 2015). VBM is a neuroimaging analysis technique that measures differences in GM and WM by dividing the brain into thousands of small regions called voxels and estimating the concentration of GM and WM in each voxel (Ashburner & Friston, 2000). DTI assesses brain connectivity by analysing the integrity and orientation of the brain's WM (responsible for transmitting brain signals from the cerebral cortex to other brain regions) by

measuring the diffusion of water in brain tissue (Ameis et al., 2011; Pina-Camacho et al., 2012).

Molecular and cellular imaging examines the biochemical activities of cells and how these are affected by pathology or injury and the effects of treatment (Asbury, 2011). These techniques use light microscopes to identify molecules by emitting light of various wavelengths to create an image contrast of target cells from other cells (Asbury, 2011).

Seibyl, Scanley, Krystal and Innis (2004) outlined three broad applications for neuroimaging technology. First, these techniques can assist in the provision of a *diagnosis* based upon the presence of abnormal brain structure or function. Second, neuroimaging can be used to assess the progression of, and treatment effects for, illness by evaluating *biological markers* (a measurable indicator of some biological state or exposure, Miquel, 2008). Third, these techniques can assist understanding of *physiology and pathophysiology* by exploring normal and abnormal brain function. Neuroimaging also provides the opportunity to assess differences between the male and female brain and has provided a sound basis establishing the extent of sexual dimorphisms in brain structure and function in typical and atypical development (Sacher et al., 2013). This may include changes brought about by the onset of progressive medical or mental illness (e.g., cognitive decline demonstrated in the onset of disease such as Alzheimer's, Ferreira & Busatto, 2011) or brain structure and function that characterises various mental disorders (e.g., cognitive impairment demonstrated in neurodevelopmental disorders such as Autism Spectrum Disorder, Bowie & Harvey, 2006).

Sexual differentiation of the brain also changes throughout development as brain structure and function are organised via the interaction of neuronal networks with their biological environment (i.e., influenced by hormones, nutrients, and other chemicals that change across the lifespan in response to various environmental and developmental influences) (Swaab, 2007). Exploring sex-based neuroanatomical differences may provide

important insights into associated behavioural differences as well as sex-specific symptom manifestation of mental disorder (Leonard et al., 2008). Structurally, sexual dimorphisms have been identified in GM and WM concentrations (den Braber, van't Ent, Stoffers, Linkenkaer-Hansen, Boomsma, & de Geus, 2013; Chen, Sachdev, Wen, & Anstey, 2007; Kanaan et al., 2012; Leonard et al., 2008) and in neuronal networks and connectivity (Ingalhalikar et al., 2013; Kanaan et al., 2012). The following section presents a discussion on the structural and functional differences that have been observed in the male and female brain using neuroimaging techniques.

3.2.2: Sex Differences in Brain Structure and Function.

Post-mortem and in-vivo studies in humans have consistently reported that the male brain is on average 11% larger than the female brain (Craig et al., 2004**;** den Braber et al., 2013). This variation has been found to exist irrespective of overall body size differences in both adults and children, where male brain volumes remain larger despite pre-pubertal similarities in height and weight characteristics (den Braber et al., 2013; Gilmore et al., 2007; Lenroot & Giedd, 2010**;** Sacher et al., 2013). For example, Witelson, Beresh, and Kigar (2006) assessed 100 brains at post-mortem and reported brain size as being minimally correlated with height, only accounting for 1-4% of the variance within each sex. Other studies however, have described relationships between brain and body size in only one sex. Nopoulos, Flaum, O'Leary, and Andreasen (2000) reported a significant positive correlation between height and cerebral volume for women but no significant association for men. In contrast, Heymsfield, Gallagher, Mayer, Beetsch, and Pietrobelli (2007) identified a significant brain size/body correlation for men but a nonsignificant correlation in women. In another study, Koh (2005) found an association between whole brain volume and body height in males and a cerebellar volume and body height correlation in females aged in their twenties. Interestingly, neither of these associations were replicated in an older aged group (individuals aged in their sixties and seventies). As Leonard et al. (2008) have suggested, such inconsistent results do not support any benefits in adjusting for physical body characteristics such as height in the study of sex-based brain differences.

While the ratio of total brain to body size does not appear to differ between the sexes, there are subtle differences in the anatomical and neurophysiological (structural neuronal connections) aspects of the cerebral organisation of males and females comparative to total brain volume (den Braber et al., 2013**;** Lenroot & Giedd, 2010), with some variation in the size of specific regions linked to variation in overall brain size (Finlay & Darlington, 1995; Leonard

et al., 2008). Larger volumes in men compared to women have been reported for most major brain regions, such as the frontal lobes, temporal lobes, left parietal lobe, insula, and cerebellum (see Figure 8 [page 59] , items 1-5, for a depiction of these brain regions) (Allen, Damasio, & Grabowski, 2002). In contrast, larger volumes in women compared to men have been reported in regions such as the frontal and medial paralimbic cortices and in the precentral gyrus, frontoorbital cortex, superior frontal and lingual gyri (see Figure 8 [page 59], items 1-5, for a depiction of these brain regions) (Goldstein et al., 2001). Findings for regional sex differences have also not been consistently reported (den Braber et al., 2013**;** Whittle et al., 2011).

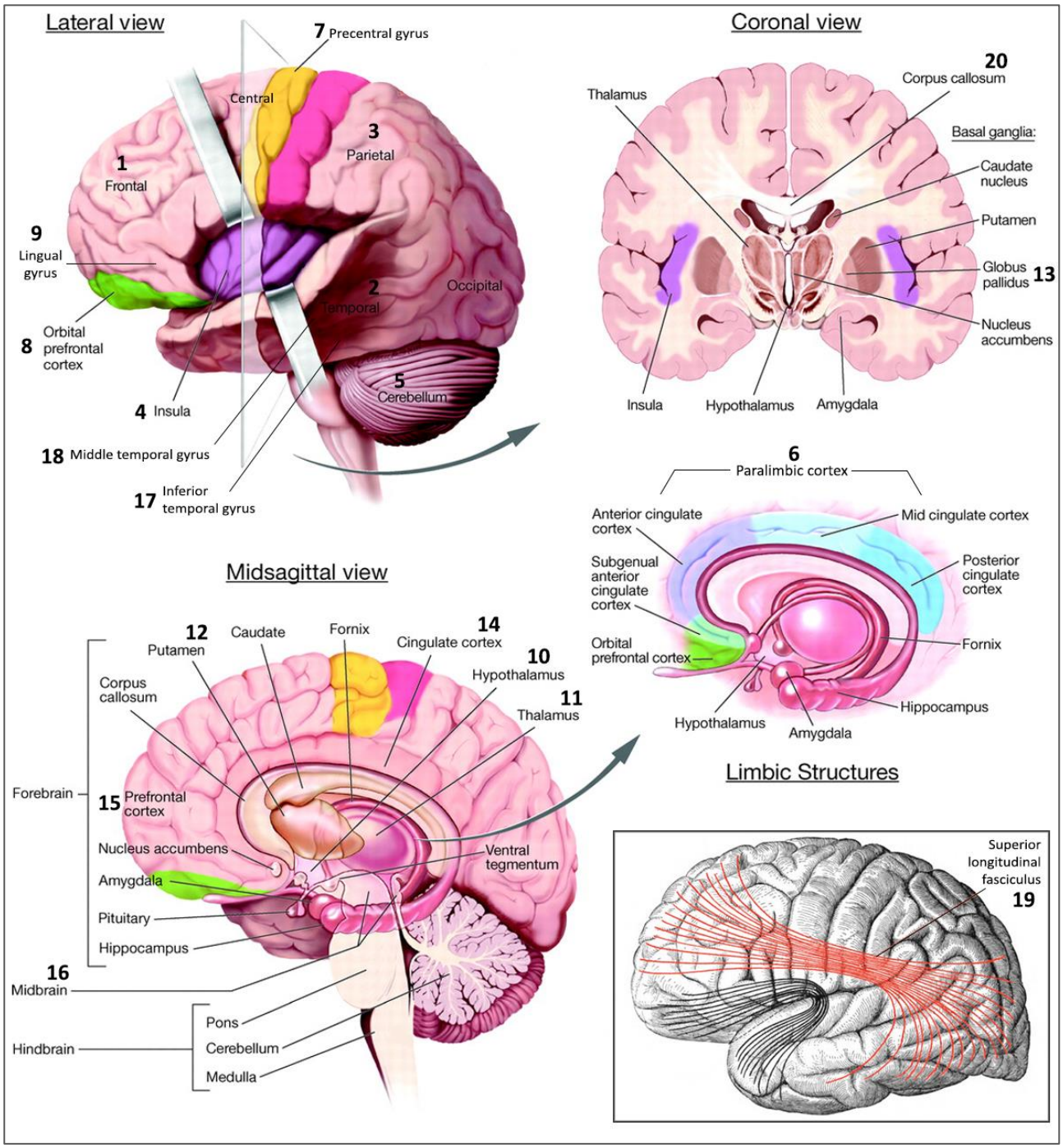

Figure 8: Overview of brain regions. Note: this figure is not a complete model of the human brain. Adapted from 'Simple Brain Diagram Labelled' by Health, Medicine and Anatomy Reference Pictures, 2013, http://healthfavo.com/simple-brain-diagram-labeled.html *Inset*: Diagram showing the superior longitudinal fasciculus (a pair of long bi-directional bundles of neurons connecting the front and the back of the cerebrum). Adapted from 'Superior longitudinal fasciculus' by Wikiwand, 2013, http://www.wikiwand.com/en/Superior_longitudinal_fasciculus#/cite_ref-4.

Interestingly, grey matter (GM) and white matter (WM) differences in male versus female brains are reported to be minimal when sex differences in overall brain size have been accounted for (Leonard et al., 2008). Therefore, in general terms, when male and female brains are of similar volume, it is highly likely they will also possess similar overall GM:WM ratios. Using MRI to study 100 men and 100 women Leonard et al. (2008) found that although men had 17% more cerebral WM and 10% more GM than women, sex differences accounted for only 1% to 5% of the variation. This apparent uniformity in brain volume and global GM and WM volumes should not deflect from the data on actual sex differences in GM and WM volumes and GM:WM ratios reported for specific regions of the brain. den Braber et al. (2013) applied MRI and DTI techniques with 40 men and 40 women and found that males had larger GM volumes for the subcortical brain region (including the hypothalamus, thalamus, putamen and globus pallidus: see Figure 8 [page 59], areas 8-9, for a depiction of these brain regions), whereas females showed evidence of larger GM volumes in areas of the cortex (including the inferior temporal, insula, cingulate, precentral and frontal/prefrontal regions: see Figure 8 [page 59], areas 1, 2, 4, and 10-12, for a depiction of these brain regions). Despite the reported male-female variation in GM *per* brain region, den Braber et al. (2013) did not report any significant differences in WM volume between the sexes for particular brain regions. Chen, Sachdev, Wen, and Anstey (2007) used MRI and VBM to analyse sex-related differences in regional GM in 184 men and 227 women with men having more GM volume in midbrain, left inferior temporal gyrus, right occipital lingual gyrus, right middle temporal gyrus, and both cerebellar hemispheres. In contrast, women showed more GM in the dorsal anterior, posterior and ventral cingulate cortices, and right inferior parietal lobule. In another study, Kanaan et al. (2012) used DTI to explore differences in fractional anisotropy (FA). Derived from DTI, FA is a metric that provides a means to assess the degree of anisotropic (the property of being directionally dependent) diffusion occurring within a region (Grieve, Williams, Paul, Clark, &

Gordon, 2007; Pfefferbaum, Sullivan, Hedehus, Lim, Adalsteinsson, & Moseley, 2000) and is a method used to evaluate white matter fibre tracts. Kanaan et al. (2012) found that men had a higher FA in WM for the cerebellum and in an area at the anterior portion of the left superior longitudinal fasciculus while women had a higher FA in the corpus callosum. Kanaan et al. (2012) proposed that higher FA is reflective of "more efficient white matter organisation" which "correlates with conduction speed" (pg. 3). Similarly, Szeszko et al. (2003) found women to have high FA in the left frontal lobe and a leftward asymmetry of the anisotropy compared to men and suggested that this may correlate with better verbal comprehension and memory functioning in women. Schmithorst, Holland, and Dardzinski (2008) reported greater FA for boys in associative white matter regions, including the frontal lobes and greater FA for girls in the splenium of the corpus callosum and suggested that boys and girls (aged 5-18 years) demonstrated differing developmental trajectories in white matter, highlighting the need to consider sex differences in similar developmental DTI studies.

The influence of age on GM and WM structure has also been posited as an important factor to consider when assessing sex differences between male and female brains. Ge et al. (2002) suggested that age-related changes in brain tissue volume in healthy individuals may have important implications in the evaluation of clinical and pathological conditions. In a study of 16 adolescents using DTI methods, Wang et al. (2012) reported significant increases in the global mean FA in boys but not in girls from 13 to 18 years of age. This suggests girls undergo earlier WM maturation whereas boys will continue to mature throughout the teen years. Similarly, De Bellis et al. (2001) studied a sample of 61 male and 57 female children and adolescents aged between 6 and 17 years and reported age-related changes in GM and WM volume. More specifically, males demonstrated greater GM decreases, WM volume increases, and corpus callosum (major WM tract that connects the left and right hemispheres, Getz, 2014) increases in comparison to females. De Bellis et al. (2001) suggested that both boys and

girls showed significant developmental changes with age but this occurred at a slower rate in girls. In a final study involving a sample of 87 males and 58 females aged 30-80 years, Hsu et al. (2008) found global decreases in FA with age in men and women and that the anterior corpus callosum, the bilateral anterior and posterior internal capsule, and the posterior periventricular regions, demonstrated the most significant age-related FA decrease. In contrast to the previous studies however, Hsu et al (2008) reported no sex-related influence to GM loss or FA decrease during aging. Age-related changes in these neuroanatomical networks that reflect gains in cognitive ability are also thought to contribute to cognitive and behavioural declines in normal aging (Ge et al., 2002; Gong et al., 2009).

Despite studies such as these, which highlight sex variations in brain anatomy and neurophysiology, there are almost as many investigations which report conflicting findings on the issue of brain-related sex differences. This inter-study disagreement might be attributed (in part) to various methodological differences, including the use of cross-sectional studies as opposed to longitudinal studies (Gong et al., 2009; Hsu et al., 2008) and the use of different types of neuroimaging techniques (Junghöfer, Peyk, Flaisch, & Schupp, 2006). This variation in methods may contribute to the inconsistent results observed in neuroimaging studies assessing sex differences and the effects of such factors as age and sex. Inter-participant brain variations, possibly due to the genetic and environmental influences those participants have experienced, may also confound results (Cosgrove et al., 2007). Continued methodologically robust studies are required to accurately identify sex-based differences in brain morphology and physiology as a means to understand the manner in which various structural differences may influence behaviour.

Further, criticism of investigations which focus on examining male-female differences in brain structure has come from researchers such as Johnson (2001) and Ngun, Ghahramani, Sánchez, Bocklandt, and Vilain (2011), who argued that identifying anatomical differences

without translating those into functional variation does not effectively advance understanding. The importance of describing functional differences in male versus female brains is beginning to be addressed in the research. For example, by utilising DTI to characterise the structural connectome (i.e., neuronal map of the brain as a whole network) in males and females, Ingalhalikar et al. (2013) found that male brains displayed greater intra-hemispheric (within brain regions) cortical connectivity, which they suggested facilitates the integration of perception and coordinated action. In contrast, female brains showed higher inter-hemispheric (between brain hemispheres) connectivity which they suggested facilitates communication between analytical and intuitive processing modes. Studies such as these are beginning to identify associations between brain form and brain function, and have implicated various brain regions in cognitive functioning, personality traits, and emotional functioning.

Chapter Four: Impacts of Sex Differences in Human Behaviour

The discussion in this chapter presents a brief review of the differences between males and females in relation to cognitive abilities, social characteristics, and personality traits plus the relative contributions of these factors to sex-differentiated behaviour (Beltz, & Berenbaum, 2013; Swaab, 2007; Takao, Hayashi, & Ohtomo, 2014). Given that the brain directs behaviour, explanations of behaviourally-based differences between men and women must incorporate the biological mechanisms and processes discussed in Chapter 2 (Section 2.2 [pages 27-38] and Section 2.3 [pages 39-43]) of this thesis. The discussion of male-female variation in behaviour will also extend to review of the key socio-cultural contexts which are believed to influence and shape sex-based behavioural differences. Of significance to this discussion is an examination of the varying impacts of biology versus socio-cultural context on male and female behaviour and this discussion will be progressed with specific reference to the research on intelligence, social behaviour, and personality in the present chapter.

4.1: Sex Differences in General Intelligence and Cognitive Abilities

Human intelligence is typically characterised by three interdependent components: verbal ability, nonverbal reasoning, and spatial ability (Mervis, Robinson, & Pani, 1999). Individual performance across these components is highly correlated as people who perform well in one component are more likely to perform well on tests which assess other components; while those who perform poorly in one area will likely also perform poorly across all areas (Gläschera et al., 2010; Mervis, Robinson, & Pani, 1999). General intelligence is a global indicator of an individual's performance and ability across these components and is assessed via a number of specific cognitive tasks. While research suggests that males and females do not differ significantly in terms of general intelligence, sex differences have been reported in the relationship between general intelligence and brain structure. Cognitive ability is influenced by neuronal factors such as neural density, functional efficiency, and integrity of brain connections, in which sex-based differences have been recognised (Burgaleta et al., 2012; Gläschera et al., 2010). In a study that examined sex differences in brain structure organisation and general intelligence, Burgaleta et al. (2012) found an overall significant relationship between intelligence for GM volume but not for WM volume. In females, GM volume correlated with general intelligence but no relationship was found for WM volume; whereas in males there were no significant correlations between general intelligence and either of these brain regions. In addition to identifying global differences in the biological correlates of intelligence, brain mapping techniques have implicated multiple interconnected regions (including the prefrontal, anterior cingulate, posterior parietal and smaller areas of the temporal and occipital cortices) with intellectual abilities (Tamnes et al., 2010). Unlike Burgaleta et al., (2012), Dunst, Benedek, Koschutnig, Jauk, and Neubauer (2014) reported intelligence-dependent WM differences only in men, where more intelligent men showed higher FA in the genu of the corpus callosum (CC) bilaterally and in the right body of

the CC than less intelligent men. In another study, Haier, Jung, Head, and Alkire (2005) used VBM to analyse MRI data and examine the relationship between sex-based structural brain variation and general intelligence in 48 participants (26 women and 22 men), demonstrating that patterns of voxel types and locations associated with intelligence differed between men and women. Overall, men had 6.5 times the number of GM voxels related to intelligence compared to women, whereas women had approximately 9 times the numbers of WM voxels associated with intelligence than did men. When considered in relation to brain region, 84% of the GM voxels associated with intelligence were found in the frontal region in women, compared to 45% in men. Further, 86% of the WM voxels identified were located in the frontal region compared to 0% in men.

The non-significant findings in global intelligence and brain structure in females versus males has led to the suggestion that sex differences may instead be due to sex-specific patterns of abilities, rather than in overall intellectual functioning (Weiss, Kemmler, Deisenhammer, Fleischhacker, & Delazer, 2003). This is supported by consistent reports of variation in performance between the sexes for various cognitive tasks (Burgaleta et al., 2012; Takao et al., 2014). Overall, males are reported to show better performance on tasks of visuospatial ability (Brooker, 2005; Gur et al, 2012; Weiss et al., 2003), such as mental rotation (Collins & Kimura, 1997; Geiser, Lehmann, & Eid, 2008; Nazareth, Herrera, & Pruden, 2013) and generating and maintaining visual images (Brooker, 2005; Loring-Meier & Halpern, 1999). In contrast, research shows that females perform better on tests of verbal ability (Galsworthy, Dionne, Dale & Plomin, 2000; Wallentin, 2009; Weiss et al., 2003), speech production (Halpern, 2012), memory (Gur et al, 2012; McGivern, Huston, Byrd, King, Siegle, & Reilly, 1997), perceptual motor skills (Ingalhalikar et al., 2013), emotion recognition (Loring-Meier & Halpern, 1999) and social cognition (Gur et al, 2012; Williams, Mathersul, Palmer, Gur, Gur, & Gordon, 2009). An exploration of all such reported differences across all of these

cognitive processes is beyond the scope of this thesis. Instead, subsequent discussion with focus on two cognitive areas for which there is strong agreement of sex-based variation, these being visuospatial abilities (favouring men) and verbal abilities (favouring women).

Visuospatial ability generally involves "representing, transforming, generating, and recalling symbolic, non-linguistic information" (Linn & Petersen, 1985, pg. 1482). In broad terms, visuospatial ability involves interpreting visual information about where object are in space. This cognitive domain is characterised by a number of different processes and thus should not be considered as unitary (Postma et al., 2000). Research findings regarding sex differences in visuospatial ability vary depending on the specific process being studied, with only a few of these processes showing significant differences (Weiss et al., 2003). The exception to this trend towards non-significance is mental rotation which has a strong research base that shows a sex difference in performance with males consistently outperforming females on tests designed to assess this cognitive ability (Geiser et al., 2008; Nazareth et al., 2013). Collins and Kimura (1997) presented 55 participants (29 males and 26 female) aged 19-33 years with a paper-and-pencil version of the Vandenberg Mental Rotations Test (MRT) and reported that male participants outscored female participants by selecting more correct answers. Parsons et al. (2004) replicated the Collins and Kimura (1997) study using the paper-and-pencil version of the MRT with a group of 44 participants (20 male subjects and 24 female subjects) aged 18 to 41 years and also reported that males outperformed the females. In a more recent replication, Geiser, Lehmann, and Eid (2008) assessed 1624 students (806 males and 818 females), also with the paper-and-pencil MRT, and found that males outperformed females across a number of specific age groups, which they classified by year starting from age nine and finishing at 23 years of age, suggesting that this trend is stable over development life stages.

While superior male performance has been identified in visuospatial abilities, females have been consistently reported to outperform men on linguistic tasks. Young girls have been shown to start speaking earlier than boys, possess larger vocabularies and read with greater accuracy and better articulation. In adulthood, women demonstrate grater speaking speeds, proficiency in word usage, fluency, and word recognition. Women are also more adept at recalling words according to specific criteria and tend to use more emotive words in text (Gawda & Szepietowska, 2013; Weiss et al., 2003). Verbal fluency is a test of executive speech (language production) and women have consistently outperformed men on tasks which tap this cognitive skill. Studies dedicated to examining sex differences in verbal fluency typically require participants to name as many words as possible beginning with a specified letter (letter fluency/lexical fluency/phonemic fluency) or belonging to a specified semantic category (category naming/semantic fluency) within a fixed time period (Shao, Janse, Visser, & Meyer, 2014; Weiss et al., 2006). Weiss, Kemmler et al. (2003) assessed verbal fluency using the Lexical and Category Word Generation Test as part of a neuropsychological battery administered to 97 university students (46 men and 51 women), with women reported to have performed better than men in both lexical and categorical fluency.

In addition to establishing possible sex-based differences in cognitive abilities, research has also begun to consider the potential underlying causal mechanisms of these differences. As will be explored in the following Section (Section 4.1.1 [pages 69-74]), research are increasingly focused on elucidating the relative influence of biological (e.g., brain structure and hormonal influences) and social (e.g., role expectations and experience) factors to the development and maintenance of the repeated cognitive variation between men and women.

4.1.1: Biological and Social Contributions to Sex Differences in Cognitive Abilities.

Sex differences in cognitive ability appear to be influenced by both biological and socio-cultural factors. Investigations into biological factors have assessed the role of genetics, hormones, and cerebral organisation (Geiser et al., 2008; Parsons et al., 2004), whereas the examination of socio-cultural influences has addressed previous exposure to tasks (experience effects), self-perception, age, and education level (Geiser et al., 2008; Wallentin, 2009; Weiss et al., 2003).

As noted in Section 2.1 (pages 23-26) of this thesis, prenatal exposure to sex hormones exerts organising effects on the developing male and female brain. Consequently, hemispheric and regional sex-based differences in cerebral organisation have been implicated in sex-based performance variations on cognitive tasks, (Jordan, Wüstenberg, Heinze, Peters, & Jäncke, 2002; Parsons et al., 2004). Sex-specific differences in brain activation across a number of cognitive tasks have been observed in various regions though the use of neuroimaging; however, not all studies have been consistent in their findings (Bell, Willson, Wilman, Dave, & Silverstone, 2006; Gabrieli, Poldrack, & Desmond, 1998; Gauthiera, Duyme, Zanca, & Capron, 2009; Weiss et al., 2003). In one fMRI study conducted by Gauthier, Duyme, Zanca, and Capron (2009) a sex effect was reported for the left inferior temporal gyrus (ITG), anterior and posterior cingulate, right anterior cingulate cortex (ACC), superior frontal gyrus (SFG), left dorsolateral prefrontal cortex (dlPFC) and lingual gyrus and the left cerebellum, with men showing significantly greater activations than women while undertaking a verbal fluency task. Similarly, Bell, Willson, Wilman, Dave, and Silverstone (2006), observed greater BOLD signal in males in the right and left dorsolateral prefrontal cortex, cingulate and right inferior parietal cortex, compared to females. In a previous study, Schlösser et al. (1998) also reported insignificant sex differences in brain activation in verbal fluency, with females showing a small area of activation in the right orbitofrontal cortex and mesial frontal cortex

that was not present in males. Males, on the other hand, were described as having a reduced response in the posterior superior temporal gyrus, asymmetry in the parietal decrement, with greater involvement in an area of the right parietal convexity, compared with the left. Weiss, Siedentopf et al. (2003) also found no overall significant activation differences between men and women while undertaking a verbal fluency task, with both sexes showing significant increases in BOLD signal in the left prefrontal cortex, in the right prefrontal cortex, in the cingulate gyrus and in the right cerebellum, although women also showed an additional significant increase of activation in the right hippocampal gyrus and hippocampus. Studies such as these demonstrate that, while similar regions are involved in the processes which underpin basic cognitive abilities, subtle differences in brain region activation between males and females may contribute to the observed behavioural differences in the completion of cognitive tasks.

In addition to sex differences in brain organisation, circulating sex hormones have also been implicated in neural activation in brain regions that mediate cognitive function (Bell et al., 2006; Maki & Resnick, 2007). As Postma et al., (2000) suggested, "separate clusters of [spatial ability] processes may be distinguished and be selectively sensitive to hormonal influences and sex differences" (pg. 564). That is, cognitive processes may be differentially affected by exogenous hormones, which in turn contribute to sex differences observed in task performance. In an interesting study that aimed to investigate the activational effects of testosterone on visuospatial abilities Aleman, Bronk, Kessels, Koppeschaar and Honk (2004) administered testosterone to a group of 26 women aged 28-32 years and had them undertake the Shepard and Metzler's three-dimensional mental rotations test. Findings indicated a causal relationship between testosterone levels and visuospatial ability as a single administration of testosterone was reported to improve visuospatial ability in young women. Findings regarding the activational effects of hormones on brain function are not uniformly

supported in the literature and conflicting results among studies suggest the need for continued investigations into this area (Cohen-Bendahana et al., 2005; Jordan et al., 2002). This inter-study disagreement was examined by Jordan, Wüstenberg, Heinze, Peters, and Jäncke (2002), who reviewed a number of neuroimaging studies assessing brain activation during a mental rotation task. Of the twelve studies identified for Jordan et al.'s (2002) review, only four addressed sex differences and each of these reported inconsistent results in relation to the relationship between areas of brain activation and cognitive performance outcomes. Although limited, such research suggests that sex-based differences in brain activation may contribute to observed differences in cognitive performance between the sexes.

The inconsistent and contradictory findings arising from investigations into proposed cognitive differences between males and females suggest that the relationship between activation patterns and cognitive performance is complex (Wallentin, 2009; Weiss et al., 2003). There are a number of considerations to be addressed when interpreting activational patterns that are associated with aspects of cognition. As Bell et al. (2006) highlighted, efficacious interpretation of activation patterns is limited in the absence of performance data. However that research team reported that, even when these data were available, differences in cognitive performance were not reflected in brain activation. Moreover, cognitive functions do not operate in isolation and better proficiency in one function may be supported by greater ability in another. Verbal fluency for example, is also influenced by verbal working memory, attention, and executive functions such as reasoning ability and perceptual speed (Salthouse, 2005; Unsworth, Spillers, & Brewer, 2011). Investigations have also implicated the involvement of the frontal cortex (specifically, the Brodmann's area, cingulate, cerebellum and Broca's area), which has also been associated with verbal fluency, verbal working memory, attentional, and executive functions (Gabrieli et al., 1998; Shao et al., 2014; Weiss et al., 2003). The extent to which regional activation can be isolated as reflective of one specific cognitive

function is difficult to establish because activation in one area may be linked to multiple cognitive abilities and contribute to activation in other areas (Gabrieli et al., 1998). This functional distribution across anatomically separate regions also requires the efficient transfer of information, which occurs via the WM microstructures linking regions (Tamnes et al., 2010). In a study that aimed to investigate the relationship between WM maturation and verbal abilities, Tamnes et al. (2010) demonstrated that verbal abilities were related to developmental differences in neuroanatomical connectivity in distributed brain regions, primarily in the left hemisphere, including the frontal and parietal areas. These results were reported to be independent of age and sex, however as previously addressed, studies have found there to be more efficient WM organisation in women (see Chapter 3, Section 3.2.2, page 57-63). Continued research is crucial in establishing the extent of biological contribution to sex-based differences in brain structure and thus understanding the observed behavioural differences in cognitive abilities between the sexes.

Investigations into environmental/socio-cultural factors (experience, self-perception of ability, age, and education level) have suggested that different cultural perspectives regarding particular cognitive abilities are likely to produce differential engagement in tasks that require those abilities (Geiser et al., 2008; Jordan et al., 2002; Parsons et al., 2004; Wallentin, 2009; Weiss et al., 2003). To explore the effects of experience on cognitive ability, Baenninger and Newcombe (1989) conducted two meta-analytic reviews of two separate but related bodies of research regarding spatial ability. The first meta-analysis concerned the relationship between spatial activity participation and spatial ability competence for males and females, with researchers attempting to determine if participation in spatial activities was positively related to scores on spatial ability measures. Baenninger and Newcombe (1989) reported a weak but statistically significant relationship between spatial activity engagement and good spatial test ability. The second meta-analysis compared male and female

susceptibility to spatial training to determine if practicing spatial tasks improved spatial test performance. Those researchers concluded that spatial training did not differentially improve the scores of males and females and that training enhanced test-specific spatial ability for both sexes but did not necessarily improve general spatial ability. That is, while males may perform better on spatial tests compared to females due to prior exposure to spatial activities in their environment (e.g., more freedom to explore their environments), spatial training (e.g., repeated exposure to stimulus materials of a specific spatial test) can be used to improve spatial ability in both males and females on a particular test but this improvement may not generalize as an application in the wider environment and practice-based improvements may not be permanent. Cultural perceptions of gender-based competencies linked to specific cognitive skills may also influence the manner in which each sex approaches cognitive tasks. As men are consistently perceived to demonstrate better spatial ability, women may feel uncomfortable with visuospatial tasks, perceive themselves at a disadvantage and respond with greater caution, with these response patterns resulting in lower scores during formal testing (Weiss et al., 2003).

Age and education effects have also been shown to influence performance on linguistic tasks (Gawda & Szepietowska, 2013; Shao et al., 2014). Mathuranath et al. (2003) reported education and age effects in a study of 153 elderly individuals, comprised of 62 males and 91 females aged between 55 and 84 years. In that study, no significant differences in performance were reported between the sexes when age and education were controlled for. Educational effects were reported to independently influence letter and categorical fluency and better performance was associated with increased education level when sex and age were controlled for. No significant performance differences were found for letter fluency but lower performance was reported for category fluency in the older age group (aged 75-84 years). This led Mathuranath et al. (2003) to suggest that letter fluency may be resistant to age effects

whereas categorical fluency may be susceptible to age-related changes. Similar results were previously reported by Troyer (2000) in a study that assessed verbal fluency in a community sample of 411 adults (288 females and 123 males) aged between 18 and 91 years. Such results suggested that, while sex *per se* may not appear to be a predictor of verbal performance, the reported findings on sex-based language trends could result from an interaction between age, education, and sex (Gawda & Szepietowska, 2013). In addition, age-related differences in cognitive performance may reflect age-related changes in brain neurology (see Chapter 3, pages 44-43) (Ge et al., 2002; Gong et al., 2009). Data such as these support the notion that assessments of sex differences in cognitive performance should seek to account for any socio-cultural features that may be confounding results.

4.2: Sex Differences in Social Behaviour: Play and Activity Interests across the Lifespan

From early development, social behaviour requires the integration of emotional, cognitive, and motivational processes with internal and external stimuli (Veenema, 2012). The postnatal period is significant for the development of social behaviour, with major changes occurring in infant responses, physiology, and morphology. Postnatal social experiences can affect brain development and the subsequent expression of social behaviour (Cushing & Kramer, 2005; Veenema, 2012), in part because it is throughout this period that learning and memory patterns (two major factors in the expression of adult social behaviour) are first established (Cushing & Kramer, 2005). Moreover, biological influences, such as previously organised sex differences in brain structure and activational reactivity to gonadal steroids, may also produce sex-specific manifestations of social behaviour (Shepard, Michopoulos, Toufexis, & Wilson, 2009).

Play is an important aspect of child social development, encouraging neurological, physiological, cognitive, and socialisation. Play allows children to explore their environments and symbolically act out feelings, thoughts, and experiences and functions as a natural form of communication between children and peers as well as with adults (Bratton, Ray, Edwards, & Landreth, 2009). The social, physical and cognitive demands of children's play have important implications for ongoing development by providing a platform from which children practice and master new skills, discover interests, and develop social relationships (Bratton, Ray, Edwards, & Landreth, 2009**;** Cooper, 2000). Researchers have acknowledged the importance of these play-based demands and examined them in relation to (i) the types of activities and interests' children engage in, (ii) the play partners children prefer to play with, and (iii) the play styles children exhibit when engaged in play. Investigations into children's play demonstrate robust and well documented sex differences in relation to all three factors and the discussion which follows outlines the major findings obtained from those investigations.

Sex differences in children's play and activity interests are associated with sex differences in other behaviours across the lifespan from early childhood to adolescence (McHale, Shanahan, Updegraff, Crouter, & Booth, 2004). In childhood, boys have been shown to be more likely to engage with transportation toys, electronics, blocks, outdoor activities, rough play, and sports (Hines, 2002; Hines, 2010; Pasterski et al., 2011; Swaab, 2007). In contrast, girls are more likely to play with dolls and doll accessories, arts and crafts, kitchen toys, and fashion and makeup (Hines, 2002; Pasterski et al., 2011; Swaab, 2007). In late childhood and throughout adolescence, these differences in play preference are reflected in variations in interests and hobbies, with boys dedicating more time to sports and girls spending more time on relationship-oriented activities and personal care (Bao & Swaab, 2010; McHale et al., 2004). This variation in toy/activity engagement has resulted in them being sex-typed as either "boys' toys/activities" or "girls' toys/activities". Finally, many of the interests and activity preferences of adolescence persist into and throughout adulthood and are differentially represented in different occupations. This results in various occupations being considered as more male- or female-appropriate because they reflect personal and social characteristics that are expected of each sex (Berenbaum et al., 2011).

In addition to sex differences in toy and activity preferences, children also begin to participate in sex-segregated play from an early age, with greater focus on same-sex versus other-sex play partners being evident from 3 to 4 years of age (Hoffmann & Powlishra, 2001; Pasterski et al., 2011). These differences increase across the childhood period (from ages 3 to 11 years, Centre for Disease Control and Prevention [CDC], 2016), during which children have been reported to play with same-sex peers as much as 11 times more often than with other-sex peers. Thes itrend is reported to change in adolescence (ranging from age 12 to 17 years, CDC, 2016), where same-sex peer networks remain stable but other-sex networks begin to develop (Fabes, Martin, & Hanish, 2003; Hoffmann & Powlishra, 2001). It has also been

suggested that the socialisation experiences children are exposed through this sex segregated play contributes to their development by impacting their interests, limiting the types of activities in which they engage, and affecting the amount of time they dedicate to particular activities (Fabes et al., 2003**;** Serbin, Moller, Gulko, Powlishta, & Colburne, 1994).

Preferences for gendered toys/activities and same-sex play partners have been attributed, in part, to differences in play styles, which are characterised by sex-specific behaviours and patterns of social interaction between boys versus girls (Berenbaum et al., 2008; Hoffmann & Powlishra, 2001; Pasterski et al., 2011). In an observational study of 203 young children (97 boys and 106 girls) that aimed to examine how children's play varies as a function of the sex of the child Fabes, Martin and Hanish (2003), found sex differences in three key areas of children's play behaviour: active/forceful play, play near adults, and stereotyped activity choice. Specifically, boys were observed more often in same-sex group play and in larger groups with established dominance hierarchies. Their play was also shown to be more unstructured, peer-oriented and peer-guided and they engaged more in active/forceful play behaviour. In contrast, girls played more in dyads that featured cooperation and verbal interaction, plus elicited behaviours that demonstrated sensitivity to a peer's needs. Their play was also more structured and adult-oriented and adult-guided. Further, Serbin, Mioller, Gulko, Powlishta and Colburne (1994) conducted a study which supported the hypothesis that boys and girls are likely to select play partners with similar play styles to their own. That research team observed 57 preschool children aged between 26 and 40 months to explore the ways in which group contexts and individual factors contributed to sex segregation in toddlers. It was found that boys and girls, in both dyadic and group play, were more likely to engage in interactive play with same-sex peers, while less social interaction was observed when children played in mixed-sex dyads. Serbin et al. (1994) suggested that this was due to

children developing particular skills, styles and social expectations that are not compatible with the opposite sex, resulting in children seeking out same-sex peers.

4.2.1: Social and Biological Explanations of Sex Differences in Play and Activity Interests.

Gendered interests are generally considered to result from socialisation, which Berenbaum et al. (2008) defined as the "process by which individuals learn about and internalise social norms" (pg. 282). Socialisation defines children's values, expectancies of success, and decisions to devote effort and time to various activities, academic fields and careers (Berenbaum et al., 2011). Socially-oriented theories suggest that children's involvement in sex-typed activities and behaviours may be influenced by socialisation processes and differential experiences imposed upon them by socialisation agents (such as parents and peer groups). Through modelling, reinforcement, extinction and other forms of behaviourally-contingent peer responses children may be encouraged to engage in the sex-typed behaviours considered appropriate for their sex (Berenbaum et al., 2008; Hassett, Siebert, & Wallen, 2008; Hines, Golombok, Rust, Johnston, & Golding, 2002; Masters, Ford, Arend, Grotevant, & Clark, 1979). Other conceptual approaches also incorporate behavioural and cognitive perspectives. For example, the theory of Behavioural Compatibility suggests that children will tend to seek out playmates whose behaviour is similar or complementary to their own. Compatibility may include similarities in interests, play styles, and social behaviour (Martin, Ruble, & Szkrybalo, 2002; Serbin et al., 1994). Cognitive developmental theories however, posit that the development of gender identity (the ability to correctly label one's own and others' gender) encourages children to seek out play partners that are similar to themselves as a means of learning more about the behaviours associated with gender roles (Serbin et al., 1994). However, it is important that socially-oriented theories be expanded to include the various biological factors that underlie socio-cultural perceptions of gender (Martin et al., 2002), and work has begun to integrate models of sex-typical and gendered behaviour (Udry, 2000).

Biological theories of sex differences in behaviour suggest that the organisational effects of prenatal sex steroid exposure as well as the activational effects of sex steroids throughout puberty induce sexual dimorphism of the underlying neural substrates of those behaviours (Berenbaum et al., 2008; Berenbaum & Beltz, 2011; Hassett et al., 2008). Organisational effects have been attributed to behavioural sex differences in childhood due to low levels of circulating hormones throughout this developmental period where differentiation is likely to be the result of hormonally-induced changes in brain structure throughout puberty (Wallen, 2005). Activational effects occur with the onset of puberty, which brings about large sex differences in the levels of circulating hormones. These effects are often dose-dependent: the amount of hormone required to masculinise or feminise behaviour varies according the behaviour being observed (Cohen-Bendahana et al., 2005) and animal studies provide evidence of the early effects of hormones on sex-typed behaviour. Wallen (2005) for example, showed that, due to the effects of prenatal androgen exposure, genetically female rhesus monkeys demonstrated more masculinized behaviour, but a reduced exposure to androgens in genetic males did not prevent masculinized behaviour (including vocalisations, play behaviours, and interest in infants). In another study that assessed toy preferences in rhesus monkeys, Hassett, Siebert, and Wallen (2008) found that male monkeys had consistent and strong preferences for wheeled toys, while female monkeys showed greater variability in preferences with no reliable inclination towards specific toys being identified. Hassett et al., (2008) concluded that the similarity of such behaviour to human children demonstrates the influential nature of hormones due to the absence of gendered socialisation.

In humans, studies of children exposed to atypical levels of prenatal hormones, resulting from disorders of sex development, also provide insight into the effects of early gonadal hormones on developing behaviour. One well researched condition is congenital

adrenal hyperplasia (CAH), a genetic disorder whereby a foetus is exposed to an excess of androgens from the eighth week of gestation due to an enzyme defect that impairs cortisol production (Berenbaum & Beltz, 2011**;** Meyer-Bahlburg et al., 2004). Increased compensatory uptake of substrate results in the overproduction of androgens because the pathway to cortisol secretion is blocked (Mathews, Fane, Conway, Brook, & Hines, 2009). The condition is usually detected and diagnosed at birth with subsequent treatment aiming to medically normalise androgen levels with cortisol replacement and to surgically feminise genitalia (Berenbaum et al., 2008; Berenbaum & Beltz, 2011). Both males and females are affected by the condition and studied behaviourally; however, research suggests greater effects in females than males, whereby the masculinizing and defeminizing effects of excess androgens are more clearly shown (Berenbaum & Beltz, 2011; Cohen-Bendahana et al., 2005).

Berenbaum and Hines (1992) studied toy preferences in 26 girls and 11 boys with CAH aged 3-8 years. Control boys and girls differed in the amount of time they spent engaged with sex-typed toys. Control boys showed greater preference for male-typed toys over female-typed toys, while control girls showed a similar preference patterns in relation to feminie-typed toys, although the trend was stronger in boys. In comparison to control girls, and similarly to control boys, CAH girls spent significantly more time engaged with male-typed toys. In contrast, there were no significant differences in sex-typed play between CAH and control boys. Girls with CAH provide the opportunity to assess the behavioural effects of prenatal androgen exposure because, despite being exposed to high levels of androgens prenatally, they are reared as females. Overall, studies show that in comparison to unaffected individuals, girls and women with CAH demonstrate more masculine-typed behaviour in a number of domains (including activity interests, personality, cognitive abilities, handedness, and sexuality) and that these results persist throughout childhood, adolescence and into

adulthood (Cohen-Bendahana et al., 2005; Mathews et al., 2009; McHale et al., 2004; Meyer-Bahlburg et al., 2004; Nordenström, Servin, Bohlin, Larsson, & Wedell, 2002).

Despite studies such as these implicating gonadal hormones as major determinants of the masculinised behaviours of CAH females, social factors are also thought to be influential. It has been proposed that parents may treat their CAH daughters in a masculinised manner due to the male-type appearance of their genitalia at birth (Beremnaum & Hines, 1992). The presence of masculine-like genitalia may also alter parent perceptions of femininity (Hines et al., 2002), which may then have an impact on the sex-typical behaviour in CAH girls through the process of socialisation. This indicates that biologically-determined sex impacts on the gendered perceptions of parents which in turn influence the behaviour of the individual. The degree to which parental perceptions influence behaviour is debated however (Craig et al., 2004) and requires further exploration (Beremnaum & Hines, 1992; Craig et al., 2004; Fagot, 1978; Hines et al., 2002). Nevertheless, this research supports the view that sex differences in the development of childhood gender role behaviour is influenced by a number of factors including social, cognitive, and biological processes (Hines et al., 2002). Therefore, biological and gendered factors must be considered in an exploration of sex differences in behaviour.

4.3: Sex Differences in Personality Traits: Aggression

Personality is defined as a set of organised psychological traits and mechanisms that influence the individual's interactions with, and adaptions to, his or her internal (psychological) and external (physical and social) environments (Larsen & Buss, 2014). Personality factors relate to enduring dispositions that guide a broad range of behaviours across a variety of situations (Ajzen, 1987) and have been explored at the individual level to identify the ways in which people are similar, and at the group level to understand the common features that make particular groups different from one another (Larsen & Buss, 2014). The study of personality is broad and complex, canvassing evolutionary, biological, psychological and social fields of research. Of particular interest is the manner in which personality differs between men and women, with each field providing different perspectives on potential sex- and gender-based differences (Krampen, Effertz, Jostock, & Muller, 1990; Weisberg, DeYoung, & Hirsh, 2011). Further, investigations of sex-based differences in aggression have featured strongly in the research and are of particular importance to discussion of personality.

Human aggression is a complex and multifaceted socio-emotional process that has been extensively investigated due to the detrimental effects it has on various social relationships (including intimate, professional, inter-group and inter-racial) as well as the material costs it creates (Gontkovsky, 2005; Krahe, 2012; Umukoro, Aladeokin, & Eduviere, 2013). Studies addressing aggression have defined it as a personality trait and have sought to capture aggressiveness by measuring it as a behavioural and/or emotional manifestation (Archer, 1991). Gender-based cultural stereotypes often portray boys and men as more aggressive and violent than girls and women in a variety of situations, with the former group engaging more often in conflict and forceful acts (Coie & Dodge, 1998; Tieger, 1980). Crime statistics obtained from a variety of countries have often been used as support for such

assertions with men overrepresented as perpetrators of violent crimes including homicide and assault (Archer & Lloyd, 2002; Bao & Swaab, 2010). Empirical studies have also indicated that males show more physically and verbally aggressive behaviour compared to women (Archer, 2004; Ostrov & Keating, 2004). Aggressive behaviour is commonly characterised according to its *motivation* and *expression* and defined by Anderson and Bushman (2002) as "any behaviour directed towards another individual that is carried out with the proximate intent to cause harm" (p. 28). There are three important aspects that characterise aggressive behaviour.

The first aspect encompasses *motivation*. This refers to a perpetrator's intent to harm or injure another person and not by the consequences of the behaviour in the absence of intent. This means that behaviour is considered aggressive if it was guided by an intention to cause harm, even if the target did not sustain any damage (Archer & Cote, 2005; Krahe, 2012). A shot fired from a gun that misses its target, for example, is still considered aggressive if the shooter intended to hit a target. In contrast, accidental harm is not considered to be aggressive because it is not intended. Receiving an injection from a doctor, for example, may result in pain but does not constitute aggressive behaviour, particularly if this action was carried out in such a manner so as to reduce the patient's experience of discomfort. The intent component is further subtyped as being instrumental or hostile. *Instrumental* aggression is premediated and refers to behaviours that are performed in order to obtain a particular goal, while *hostile* aggression (also referred to as reactionary aggression) is conceived as an impulsive or unplanned reaction to a perceived provocation driven by an aggressor's desire to express negative feelings such as anger (Anderson & Bushman, 2002; Krahe, 2012). An example of instrumental aggression would be taking an individual hostage in order to secure a ransom, while an example of hostile aggression would be to strike another individual out of anger following provocation.

The second aspect focuses on understanding the *consequences* of aggressive behaviour. An individual committing an aggressive act must have awareness that the behaviour has the potential to cause harm or injury to another. Hitting another person out of anger or frustration is likely to result in physiological harm (e.g., bruising, swelling, and soreness) and would thus be considered aggressive whereas, instances of carelessness or incompetence that result in an injury do not constitute aggressive acts due to a lack of intent. Someone slipping on a wet floor that was not signed for example, would not classed as a victim of aggression if the cleaner did not intend for that person to slip and injure themselves.

The third aspect relates to the *reaction* of the target individual and dictates that some form of avoidance behaviour must follow the onset of aggression. This means that any individual who is the target of aggressive behaviour would be motivated to avoid that behaviour (Anderson & Bushman, 2002; Krahe, 2012). A target stepping backwards to avoid being hit by an aggressor is an example of this reaction based on the understanding that the behaviour is likely to cause harm.

The manner in which aggression is expressed can be classified as being direct or indirect. *Direct* aggression involves physical or verbal forms that occur in a confrontation between an 'aggressor' and a 'target', whereas *indirect* aggression (also referred to as relational or social aggression, Archer & Coyne, 2005) is covert and devoid of contact with the victim. This latter type of aggression aims to damage the victim's self-esteem or social status, through such behaviours as gossiping, spreading rumours, and social exclusion and does not occur in the presence of the target (Card, Stucky, Sawalani, & Little, 2008; Krahe, 2012; Lundh, Daukantaité, & Wångby-Lundh, 2014). Males have been shown to engage more in direct aggression than females and indirect aggression is believed be more characteristic of girls than boys (Card, Stucky, Sawalani, & Little, 2008). In a meta-analysis aimed at assessing sex differences in physical, verbal, and indirect aggression, Archer (2004) reported that men

showed more physical and verbal aggression. However the sex difference for verbal aggression was smaller than that for physical aggression. In contrast, no sex differences were found between the sexes for indirect aggression possibly indicating that females did not engage more in this behaviour than males.

Contextual factors may also affect the magnitude of sex differences in aggression. Björkqvist (1994) highlighted that that it is important to distinguish between the (i) dynamics of the conflict (i.e., groups versus interpersonal) and (ii) sex of the opponent (male-male, female-female, or male-female) as these factors are likely to impact sex-based differences in aggressive styles. Moreover, research findings have been mixed depending on methodological issues including the type of reporting (i.e., observation versus self-reporting), operational definitions of aggression that reflect male-typical patterns, and the age the aggressor (Archer, 2004; Archer & Coyne, 2005; Björkqvist, 1994; Rhee & Waldman, 2002). Such factors were explored by Ostrov and Keating (2004) in an observational study assessing the aggressive tactics used by preschool children during free versus structured play. Under both play conditions, girls exhibited higher rates of relational aggression than boys whereas boys showed more direct aggression (including physically and verbally aggressive behaviours) than did girls. Interestingly, the identified sex differences were smaller in structured play compared to free play: rates of physical aggression in boys declined and verbal tactics increased when interacting with other boys, while girls continued to use relational aggressive tactics when interacting with other girls. These differences were attributed to the presence of an adult, indicating that boys and girls possibly altered their interactional styles to reflect the social context – in this case, the presence of an adult supervisor. Ostrov and Keating (2004) also considered playmate sex and method of measurement (i.e., teacher reports, peer nomination, and behavioural observation) as part of their analysis of 48 children (24 males and 24 females) aged from 51 to 77 months. In regards to playmate sex, they found that

aggressive styles were independent of this factor, with the exception of physical aggression where boys directed more physical aggression toward other male playmates than they did toward female playmates. When considering the measurement of aggression, it was found that teacher's reports of boys' and girls' aggressive styles accurately reflected observational data, again with the exception of physically aggressive behaviours, which were attributed to gender stereotypes that resulted in boys' acts of physical aggression being considered as 'roughhouse' play and girls' physical aggression into unintentional actions. Explanatory investigations of human aggression have encompassed evolutionary, biological, and social perspectives in an effort to provide a multifaceted understanding of this complex and contextually-driven behaviour pattern. Section 4.3.1 (pages 88-98) of this thesis presents a review of these investigations with specific reference to the underlying biological and social processes that might drive sex differences in human aggression.

4.3.1: Multifaceted Explanations of Sex Differences in Aggression.

Various explanatory theories, incorporating the perspectives of a number of disciplines (psychological, behavioural, social, evolutionary, and biological) have been proposed to explain the development of aggression across the lifespan (Krahe, 2012; Krahè, 2013). These theories differ in their explanations of causality but are complementary in that they emphasise different aspects of aggression as a complex social behaviour. The evolutionary perspective suggests that men and women have developed sex-specific biological mechanisms and thus occupy sex-specific social roles. Derived from these social roles are the types of behaviours considered appropriate for an individual based on their actual or perceived sex. Sociological theories explore how the social environment interacts with biological factors (Krahe, 2012). The following discussion explores these theories as interconnected explanations, highlighting the aspects of each that influence the development and manifestation of aggressive behaviour in men and women.

Evolutionary explanations, which have been predominately assessed in animal model studies in various species, attribute the origin of sex differences in aggression to the principle of sexual selection, a mode of natural selection used to describe those forces that act differentially on the two sexes to increase the fitness of each sex in relation to *reproduction* by promoting sex-limited traits as a means of competing for (a mechanism termed 'intra-male competition') and choosing mates (a mechanism often referred to as 'female mate choice') within a species (Berns, 2013; Dunham, Maitner, Razafindratsima, Simmons, & Roy, 2013). Craig and Halton (2009) suggest that higher levels of aggression in males provides them a competitive edge in securing resources (i.e., females with which to mate) through combat and in establishing dominance hierarchies with respect to reproductive success. That is, the more aggressive an individual is, the more likely they are to mate and pass on their genes via their capacity to control access to mating partners, which results in the favouring of aggressive

behaviour through natural selection (Krahè, 2013). Findings from the work of Trivers (1972) and Clutton-Brock & Vincent (1991), Archer (2009) further elaborate this position by incorporating the principles of parental investment (the investment of resources by parents in order to raise offspring and the risks associated with protecting them) and differential reproductive rates (the rates at which males and females are able to mate again after producing offspring). Archer (2009) explains that higher rates of aggression in males can be attributed to their lower parental investment which increases their capacity to secure a greater number of mating partners. In contrast, due to their higher parental investment (including the specialisation of gametes and rearing offspring to ensure that offspring's survival), females become a limiting resource for males when they exert choice, or preference, in the selection of mating partners. It is more beneficial then, for males to seek access to multiple mates, which drives intra-male competition (Archer, 2009). Campbell (1999) attributed women's engagement in indirect forms of aggression to their greater parental investment suggesting that females have increased motivation to ensure their offspring's survival for two reasons. First, internal fertilisation means that the mother is guaranteed that her offspring will carry half her genes and this increases her motivation to ensure the offspring's survival. Males cannot be certain that mating will result in an offspring that carries their genes and will therefore seek multiple mating partners to increase their chances of producing offspring. Second, offspring are initially more reliant upon the mother for care through lactation. In both cases, a female's engagement in more direct forms of aggression, which puts the perpetrator and target at greater risk of harm or injury, would potentially diminish her capacity to provide for her offspring, necessitating other, less risky forms of aggression. Similar trends have also been observed in species where males exert mate choice and females compete for access to mates. Sexual selection theory has also been attributed to sex differences in human aggression and used to explain higher rates of (i) direct physical

aggression in males compared to females and (ii) same-sex aggressive encounters (i.e., between males) (Archer & Cote, 2005). Daly and Wilson (1990) applied sexual selection theory to human homicide and considered the higher rates of male intra-sex homicide and the concentration of these among men with fewer resources as indicative of sexual selection. From the evolutionary perspective, aggression is functional in that it facilitates mating success and sexual selection is the mechanism by which sex differences in aggressive behaviours arise.

Sociological explanations contribute to understanding of sex differences in aggression by suggesting that men and women have distinct social positions and undergo differing psychological processes in adjusting to their predetermined social roles (Eagly & Wood, 1999). Therefore, differences in aggressive behaviour may instead be a response to the gendered roles that men and women subscribe to (Richardson & Hammock, 2007). That is, the male gender role is associated with assertiveness and dominance, which facilitate aggression, while the female gender role is associated with characteristics such as nurturance and empathy, which demote aggressiveness (Krahè, 2013; Richardson & Hammock, 2007).

Behaviourally oriented research indicates that aggressive behaviour is acquired as a result of an individual's experiences and the process of socialisation (Björkqvist, 1994; Krahè, 2013). According to *social learning theory* behaviour is learned either by direct experience or via observational learning and reinforced either through direct reinforcement (by achieving a desired goal or receiving social approval) or observational learning (by acquiring behaviour by watching others being reinforced for their aggressive behaviour) (Krahe, 2012; Szeszko et al., 2003). This suggests that sex differences emerge due to the differential reinforcement men and women receive when they engage in aggressive behaviour (Krahe, 2012). Anderson and Bushman (2002) suggest that learning theories are particularly useful in explaining instrumental aggression, which involves behaviours considered to be overt, easily observed, and goal directed. Traditional behavioural theories of aggression have been redefined to

include cognitive factors with the potential to elaborate o the association between inner experiences (e.g., attitudes) and overt actions (i.e., aggression). Campbell, Sapochnik, and Muncer (1997) discussed the concept of social representations and aggression in men versus women. These researchers describe social representations as "cognitive packages containing attributions of cause, attitudes and images of a phenomenon that are transmitted socially within a cultural and which are both generative and interpretive of behaviour" (pp. 162). Campbell et al., (1997) apply social representations to the types of aggression that men and women are more likely to engage in by suggesting that women hold more expressive representations, in which aggression is negatively viewed as a loss of self-control, while men to an instrumental representation, in which aggression is positively viewed as the exercise of control over others. Numerous studies using the Expagg, a psychometric measure developed to measure people's social representations of aggression, have supported this view (Archer & Haigh, 1997; Campbell & Muncer, 1994; Campbell, Muncer, Guy, & Banim, 1996; Campbell, Sapochnik, & Muncer, 1997). These representations reflect how men and women feel about aggressive incidents once they have occurred and influence behaviour because they relate to outcome expectancies (Archer & Haigh, 1997; Tapper & Boulton, 2000).

Socio-cultural expectations of what constitutes appropriate behaviour for men and women are in part influenced by the biological underpinnings of that behaviour. Biological approaches to understanding aggressive behaviour address the roles of genes, hormones and brain mechanisms, as well as the interactional nature of these factors on the development and manifestation of sex differences in aggression. Evidence for potential genetic underpinnings in aggression has been derived from twin and adoption studies that have aimed to determine the extent to which individual differences can be linked to genetic or environmental factors (Krahe, 2012; Miles & Carey, 1997). Twin studies have compared monozygotic (MZ) and dizygotic (DZ) twins, which share 100% and 50% of their segregating genes respectively. A

characteristic is considered as genetically influenced when MZ twins are more similar than the DZ twins. That is, the higher the correlation for MZ twins compared to DZ twins, the greater the level of heritability for a characteristic (Malouff, Rooke, & Schutte, 2008). Adoption studies compare individuals to their biological parents (shared genes) and their adoptive parents (shared environment) to separate genetic and environmental influences (Miles & Carey, 1997). Higher correlations between adoptees and their biological parents compared to the correlation of the adoptees and their adopted parents indicate greater heritability for a characteristic (Malouff, Rooke, & Schutte, 2008).

In a meta-analysis comparing 24 twin and adoption studies assessing genetic and environmental contributions to antisocial behaviour, Miles and Carey (1997) concluded that both factors are responsible for individual differences in aggression and that heritability accounts for up to 50% of the overall variance. Interestingly, heritability was slightly more influential in males than females, while common (shared) environment was more important in females than males. Shared environmental influences are defined as being experienced similarly by individuals, thus making family members alike, and are contrasted with non-shared environmental influences, which refer to those unique experiences that make family members different from one another. Rhee and Waldman (2002) conducted a meta-analysis of 51 twin studies in order to determine the relative magnitude of genetic and environmental influences on antisocial behaviours. Their findings suggested moderate genetic (additive and non-additive) and environmental (shared and non-shared) influences. Additive genetic influences indicate that the effects of alleles from different loci are independent and add up to influence the liability of a trait. Liability collectively refers to genetic and environmental factors that contribute to the development of multifactorial traits. Multifactorial traits are in turn described as traits that result from the interaction of one or more environmental factors and two or more genes (Cummings, 2014). In contrast, non-additive influences refer to alleles

that interact with each other to influence the liability for a trait, either at a single locus (i.e., dominant allele) or at different loci (i.e., epistasis - effect of one gene masking the phenotypic effects of a different gene; Brooker, 2005). The Rhee and Waldman (2002) meta-analysis, no statistically significant sex differences were found, suggesting that the magnitude of genetic and environmental influences on antisocial behaviour in males and females is similar. In response to inconsistencies reported across studies, Rhee and Waldman (2002) also examined the effects of a number of moderating factors including operationalisation of antisocial behaviours, use of different assessment methods, zygosity determination method and participant age. All of these factors were found to account for significant differences in the genetic and environmental influences on antisocial behaviour, leading Rhee and Waldman (2002) to recommend that future research encompass examinations of these moderating effects in order to better understand genetic and environmental influences and the potential interactions between these factors. That is, individual circumstances and experiences may also contribute to a greater expression of genetic predispositions for aggression (Popma et al., 2007). Overall, studies suggest a genetic contribution to aggressive behaviour; some genes and some environmental factors may account for differences both within and between the sexes but variability among twin and adoption studies has made interpretations regarding the magnitude of these contributions difficult (Rhee & Waldman, 2002).

Further evidence of genetic contributions to aggressive behaviour comes from animal studies that aim to determine the effects of sex chromosome complement and gonadal secretions and the potential interaction between these two factors. Using the FCG mouse model for example, Gatewood et al. (2006) assessed the potential for sex differences in aggressive and parental behaviours in mice arising from the different complements of sex-linked genes on the X and Y chromosomes. Measures of aggression included (i) the latency in onset of aggression and (ii) the proportion of mice that displayed aggressive behaviours

(including biting, chasing, lunging, or wrestling) towards an intruder mouse. Significant differences (independent of hormonal influences and the *SRY* locus) were only noted between XX and XY^- females, where XY^- females behaved in a similar manner to gonadal males indicating that gene(s) on the sex chromosomes, other than *SRY* influence aggression. It is difficult however, to attribute the effects to genes on either chromosome specifically and further research is required to identify X- and Y-linked genes that may be involved in aggression (Gatewood et al., 2006). While an increase in aggressive behaviours in the XY^- females compared to XX females suggests a sex chromosomes influence, similar levels of aggressiveness between gonadal males (and between gonadal males and gonadal females with the XY sex chromosome complement) indicate that gonadal hormones also affect aggressive behaviour. Despite these possible X and/or Y chromosome contributions however, it appears that the underlying aetiologies of aggressive behaviour are possibly similar for males and females and that additional biological and social experiences may account for the reported sex difference in levels of aggression favouring males (Tuvblad & Baker, 2011).

Investigations assessing hormone-aggression relationships have primarily focused on the role of hormones across the lifespan. Animal studies have consistently shown associations between the gonadal hormone testosterone and aggressive behaviour but research examining such a relationship to human aggression has been inconclusive (Archer, 1991; Book, Starzyk, & Quinsey, 2001; Popma et al., 2007). There are several factors within the various studies that may contribute to inconsistent findings on the effects of testosterone on human aggression. Methodological differences in factors such as participant age, time of day for testosterone measurement, broad definitions of aggressive behaviour, assessment of aggression as a personality trait versus overt behaviour, and use of behavioural observation versus self-report methods to establish aggression levels have made comparisons between and across studies difficult, even when meta-analytic techniques are employed (Book et al., 2001). In

addition, further factors such as context (i.e., responses to antecedent events) and cyclic fluctuations of testosterone are likely to elicit variable and possibly confounding individual responses across participant groups (Book et al., 2001). Nevertheless, human studies have typically focused on the increasing levels of testosterone during puberty due to the rapid changes in behaviour that occur throughout this developmental period (Ramirez, 2003). While some studies have shown that increased levels of exogenous testosterone are associated with greater manifestations of aggression in males (Kouri, Lukas, Pope, & Oliva, 1995; Pope, Kouri, & Hudson, 2000) others have reported weak or non-existent correlations (Archer, 1991; Book et al., 2001; Rowe, Maughan, Worthman, Costello, & Angold, 2004). In a study of 15-17 year old males, Olweus, Mattsson, Schalling, and Low (1988) concluded that testosterone had causal effects on provoked and unprovoked aggressive behaviour, and suggested that increased levels of circulating testosterone resulted in a tendency to respond aggressively to provocation. Finkelstein, Von Eye and Preece (1994) undertook a longitudinal study of males and females aged 9-11 years at study onset with data being collected at three time points over a 6 year period (1983: 63 girls and 43 boys; 1985: 48 girls and 29 boys; and 1987: 40 girls and 30 boys). It was reported that despite early differences with boys self-reporting greater aggressiveness, these sex differences had disappeared by late puberty. Finally, Halpern, Udry, Campbell, and Suchindran (1994) conducted a longitudinal study of 100 males undergoing puberty and found little evidence of a relationship between aggression and testosterone levels.

Studies of naturally occurring endocrine conditions involving hormone abnormality have also provided some insight into the androgenic influences on aggressive behaviour but results have also been inconclusive. Studies of girls with CAH for example, have reported increased levels of aggression compared to unaffected female counterparts when aggression has been measured behaviourally or as a personality trait (Berenbaum & Resnick, 1997;

Mathews et al., 2009; Pasterski et al., 2007). Idris, Chandran, Zakaria, & Rasat (2014) found no differences between CAH girls and community and control girls aged 6-18 years old for aggression measured by the Child Behaviour Check List (CBCL). In contrast, Berenbaum and Resnick (1997) assessed aggression in children, adolescents, and adults with CAH using the Multidimensional Personality Questionnaire (MPQ) (a personality measure with an aggression subscale) and the Reinisch Aggression Inventory (RAD) (a measure of an individual's potential for aggressive behaviour) and reported adolescent and adult females with CAH as having higher scores than control females on both measures. These results suggest that early androgen exposure may increase self-reported aggression in female adolescents and adults but not in children. Non-significant findings for the child group led Berenbaum and Resnick (1997) to suggest that it is not possible to determine whether early androgens have smaller effects on aggression in childhood than in adolescence and adulthood.

It may be that the biochemical mechanisms underpinning aggressive behaviour elicit higher responsiveness of the neural circuits related to aggression (de Almeida, Cabral, & Narvaes, 2015; Hermans, Ramsey, & van Honk, 2008). In males, the presence of testosterone during critical developmental periods acts on the prenatal organization of cortical and subcortical pathways that mediate aggression and, due to changes in the organisational structure of the brain during puberty, the subsequent activational effects of various neurotransmitters and hormones contribute to sex differences in aggressive behaviour (Tieger, 1980). Therefore, sexual dimorphisms in the activation of brain regions may also contribute to sex-based differences in aggressive behaviour. However, more research is required in order to establish and better understand the links between aggression and the interconnected biological factors underpinning such behaviour. Testosterone for example, has been shown to elevate the expression of the vasopressin gene in the amygdala, which has been implicated in social behaviour (Montoya, Terburg, Bos, & van Honk, 2012). Further, the

distribution of vasopressin and vasopressin receptors found throughout the neural networks that mediate social cognition can vary depending on hormonal state, sex and social experience (Albers, 2012). As reviewed by Gontkovsky (2005) dopaminergic transmission through mesolimbic and mesocortical regions of the brain may influence the expression of aggressive behaviour, while serotonergic neuronal projection originating in the brainstem and ascending to brain other structures (including the substantia nigra, hypothalamus, thalamus, and basal ganglia as well as other areas of the cortex) has also been implicated in the mediation of aggression. It has also been suggested that cortisol mediates the relationship between testosterone and aggression (Popma et al., 2007; Terburg, Morgan, & van Honk, 2009). In a study of 12- to 14-year-old male adolescents, Popma et al (2006) found a significant positive relationship between testosterone and overt aggression in subjects with low cortisol levels but not in those subjects with high cortisol levels leading them to suggest that the relationship between testosterone and aggression is mediated by cortisol. Terburg, Morgan, and van Honk (2009) furthered this position through their exploration of the interactional nature of the hypothalamus-pituitary-adrenal (HPA) axis and the hypothalamus-pituitary-gonadal (HPG) axis. Activated during stressful events, the end product of the HPA axis is cortisol, which assists in restoring the body to a homeostatic state following a stress-response (Kudielka & Kirschbaum, 2005; Terburg et al., 2009), while the activation of the HPG axis, which is involved in sexual maturation at puberty as well as the reproductive and immune systems, results in the production of sex steroids at the gonads, including testosterone in males and oestrogen and progesterone in females (Terburg et al., 2009). As both testosterone and cortisol bind to steroid-receptive centres in the brain, the interactional nature of these axes comes from the inhibitory effects that one end product has on the other axis; that is, testosterone inhibits HPA axis functioning while cortisol impedes HPG axis activity.

Sex differences in HPA axis responses may be due to the circulating steroid hormones and corticosteroid binding globulin levels and sexual dimorphisms in brain function in those areas that mediate the processes of psychological stress and emotional processing, including the prefrontal cortex, the hippocampus, and amygdala, and thus aggressive behaviours (Goel, Workman, Lee, Innala, & Viau, 2014; Kudielka & Kirschbaum, 2005; Peper et al., 2010; Terburg et al., 2009; Uhart, Chong, Oswald, Lin, & Wand, 2006). Such research suggests that hormonal factors do not act in a deterministic manner, but rather interact with additional factors in the social environment (such as relevant environmental stimuli, Book et al., 2001) to shape aggressive behaviour (Björkqvist, 1994).

Chapter Five: Sex Differences in the Onset and Development of Mental Disorder

This discussion explores the major findings from studies which have investigated sex differences in the development of mental disorder and advances the theme of consistently-reported disparity between men and women in the prevalence rates of sub-types of mental and behavioural disorder (Bao & Swaab, 2010; Eaton et al., 2012; Kistner, 2009). Sex-based variation is also reported to be evident in factors such as risk and susceptibility for disorder onset, progression once a disorder occurs, manifestation of symptoms, treatment-seeking behaviour and treatment responsiveness, and subsequent psychological adjustment to the impacts of mental disorder on daily functioning and performance (Bao & Swaab, 2010; Wizemann, & Pardue, 2001; World Health Organisation [WHO], 2015). Clinical studies show that females have higher prevalence rates of depression (2:1, Kuehner, 2003), anxiety disorders (2:1, Leach, Christensen, Mackinnon, Windsor, & Butterworth, 2008), and eating disorders (10:1, American Psychological Association [APA], 2013) than males. Conversely, there is higher representation of males for substance use disorders (3:1, Greenfield, Back, Lawson, & Brady, 2010), Attention Deficit Hyperactivity Disorder (4:1, Ramtekkar, Reiersen, Todorov, & Todd, 2010) and antisocial personality disorder (3:1, Alegria et al., 2013). These reported differences in prevalence rates suggest that the manner in which mental disorder is experienced and expressed in daily life is possibly impacted by an individual's sex and/or gender, thus highlighting the importance of considering these basic variables when conducting clinical evaluations of and treating mental disorder (Afifi, 2007; Pankevich et al., 2011; WHO, 2015).

5.1: Overview of Depression and Anxiety Disorder: Characteristics and Prevalence

Disorders of mood (e.g., Major Depressive Disorder, Disruptive Mood Dysregulation, Persistent Depressive Disorder [Dysthymia]) and anxiety (e.g., Generalised Anxiety Disorder, panic disorder [PD], specific phobia [SP], and post-traumatic stress disorder [PTSD[1]]) are among the most commonly-occurring mental disorders (Bekker & van Mens-Verhulst, 2007; Lewinsohn, Goltib, Lewinsohn, Seely, & Allen, 1998) in Western societies and, due to this, will become the focus for the remainder of the discussion on sex-based differences in this chapter of the thesis. The 2007, it was reported in the National Survey of Mental Health and Wellbeing that mood disorders affected approximately 6% of the Australian population aged 16-85 and anxiety disorders affected approximately 14% of all people in the same age bracket. These figures were derived from data provided by participants who reported on their mental health in the 12 month period prior to completion of the survey (Australian Bureau of Statistics [ABS], 2009). A higher proportion of females than males reported experiencing mood disorders (i.e., 32% and 20% respectively) and anxiety disorders (i.e., 18% and 12% respectively) in their lifetime (Australian Bureau of Statistics [ABS], 2009). These prevalence rates have been replicated across ethnic groups (Altemus, 2006; Nemeth, Harrell, Beck, & Neigh, 2013; Sagud, Hotujac, Mihalhevic-Peles, & Jakovljevic, 2002) and contribute significantly to the global burden of disease (World Health Organisation [WHO], 2008). The WHO (2015) for example, predicts that depression will be the second leading contributor to the global burden of disease by the year 2020.

Depressive disorders are characterised by the presence of sadness and/or loss of interest or pleasure and are accompanied by cognitive (e.g., poor concentration, indecisiveness) somatic (e.g., fatigue, weight loss/gain) and affective (e.g., feelings of

[1] Although classified in the DSM-5 as a Trauma- and Stressor-Related Disorder, PTSD is included in this discussion according to previous diagnostic criteria classifying it as an anxiety disorder as research concerning this disorder has been based on these previous diagnostic criteria.

worthlessness, guilt) changes that result in significant functional impairment in a number of domains, including occupational and social (APA, 2013). The various subtypes of depression are differentiated on the basis of time of onset, duration, or presumed aetiology (APA, 2013). Major depressive disorder (MDD), most often referred to as simply "depression", represents the most commonly-diagnosed depression subtype. The research focused on mapping sex-based differences in this condition across the lifespan suggests that such variation becomes evident in adolescence (Davies & Wilkinson, 2006; Getz, 2014). Epidemiological studies of depression often report no sex differences in childhood, with an overall sex ratio of 1:1 (Abel & Kulkarni, 2006). Instead, starting sometime around menarche, at about age 12-13, rates of depression in girls begin to increase while rates in boys remain stable or increase to a lesser extent (Hankin, Wetter, & Cheely, 2008; Nolen-Hoeksema & Hilt, 2009). By late adolescence, girls are twice as likely to be diagnosed with depression than boys (Abel & Kulkarni, 2006). This observed sex-based disparity in prevalence rates has been attributed to a number of factors involving women having more depression first onsets, longer depressive episodes, and a greater recurrence of depression. It has also been suggested that there is also a tendency for professionals to diagnose depressive disorders in females more readily and women are more likely to seek help for depressive symptoms (Abel & Kulkarni, 2006; Gijsbers van Wijk, Huisman, & Kolk, 1999; Klose & Jacobi, 2004; Nolen-Hoeksema & Hilt, 2009; WHO, 2015).

Anxiety disorders are characterised by excessive fear and anxiety which contribute to behavioural disturbances. *Fear* refers to an emotional response to a real or perceived and immediate threat and is associated with physiological arousal necessary for responding to that threat, thoughts of immediate danger, and escape behaviours (APA, 2013). *Anxiety* is the anticipation of a (real or perceived) future threat and is associated with symptoms such as muscle tension, vigilance in preparation for future danger, and avoidance behaviours (APA, 2013). An anxiety response is preceded by an anxiety-provoking event or group of events,

which are used as the basis of subtyping anxiety disorders. Specific phobia, for example, refers to a marked anxiety brought on by the presence of a particular stimulus, such as animals (e.g., spiders, dogs), natural environments (e.g., heights, storms, water), or situational factors (e.g., aeroplanes, elevators). Social phobia refers to marked anxiety about social situations where an individual is likely to be scrutinised by others. Generalised Anxiety Disorder (GAD) is characterised by excessive anxiety about a broad range of often-undiscernible events that results in worry that is disproportionate to the actual impact of the anticipated event (APA, 2013). Understanding sex-based differences in the manifestation of anxiety symptoms is complicated because anxiety-provoking events vary greatly from one person to the next. Thus, a detailed exploration of differential symptom manifestation between the sexes for each anxiety disorder is beyond the scope of this chapter. Instead, discussion of the biological underpinnings of anxiety will address this condition in general and consider specific sex-based differences among the various subtypes only when these have appropriate evidential support. Disparate prevalence rates show women are up to 85% more likely to be diagnosed with an anxiety disorder than are males. The vulnerability of females versus males for developing a pathological anxiety is consistent across the anxiety subtypes including PD (3:1), agoraphobia (4:1), SP (2:1), GAD (3:1), and PTSD (3:1) (Afifi, 2007; Carmen, McLean, Asnaani, Litz, & Hofmann, 2011). Despite these reasonably consistent sex-differences in prevalence rates, sex-based variation in the manifestation of anxiety symptoms remains unclear and requires further research (Bekker & van Mens-Verhulst, 2007; Carmen et al., 2011; Leach et al., 2008; Lewinsohn et al., 1998).

In addition to this sex-based variation in prevalence rates of mental disorder, differences have also been reported in presentation of symptoms and response to treatment in men and women diagnosed with depression and anxiety disorders (Piccinelli & Wilkinson, 2002; Sagud et al., 2002). As these two disorders are highly prevalent, plus comorbid, and

show clear female bias in incidence, and they provide a sound basis for in-depth exploration of the underlying factors which might contribute to differing experience and manifestation of depression and anxiety symptoms between the sexes. Understanding these differences has important implications in the development of effective prevention measures and treatment plans and further research is required to ensure the appropriate provision of care (Carmen et al., 2011; Nemeth et al., 2013).

5.2: Influence of Gendered Factors on Prevalence of Depression and Anxiety Disorders

Researchers (Klose & Jacobi, 2004; Hankin et al., 2008; Piccinelli and Wilkinson, 2000) recommend that investigations that seek to explain the male-to-female prevalence disparity in depression and anxiety must also examine sociodemographic and gender-related factors to illuminate the social/environmental issues which might (in addition to biological factors) contribute to this disparity.

When considering the effects of age for example, the onset of depression for males and females occurs at similar ages with onset at a younger age being predictive of a greater number of depressive episodes. However, these trends in age of depression onset and number of subsequent depressive episodes apply to the course of depression symptoms only in females (Essau, Lewinsohn, Seeley, & Sasagawa, 2010; Klose & Jacobi, 2004). Further, it appears that pubertal stage as opposed to chronological age is more predictive of the onset of depressive symptoms in young girls (Afifi, 2007; Kuehner, 2003) and is reflected in rates of depression being shown to increase significantly for women from mid-puberty, during and after pregnancy, and during menopause (Nemeth et al., 2013). Sex-based differences have been attributed to interactions between biological and social factors. From the biological perspective, hormonal fluctuations during these maturational periods alter brain structure and function, thus contributing to sex based differences in experiences of depression (Altemus, 2006). Socially, female adolescents report having a greater number of interpersonal stressors concerning peers, romantic associations, and family relationships whereby affection, closeness, social validation and acceptance are tied to self-definition and identity and thus emotional well-being (Hankin et al., 2008; Natsuaki et al., 2009). Adolescence has been characterised as a challenging stage of development during which youth face normative stressors within their relationships, which may mediate elicit differing male/female social pressures and coping styles that mediate, at least in part, the sex difference in adolescent

depression (Hankin et al., 2008; Hankin, Mermelstein, & Roesch, 2007; Slatkin, 1984). These stressors, coupled with biological maturation (such as the previously mentioned alterations in brain structure and function), highlights the necessity of exploring socially-derived influences on sex differences in depression.

In a study that aimed to examine sociodemographic factors potentially associated with gender differences in mental disorder, Klose and Jocobi (2004) used general population data from 4081 men and women aged between 18 and 65 years. They reported that the emotional advantages or disadvantages of marital status, employment status, number of children, parenthood and social class generally applied equally to men and women diagnosed with mental disorder, including depression and anxiety. Piccinelli and Wilkinson (2000) reported an absence of gender-specific differences in participant's recollections of past depressive episodes and levels of social support, but did find that females were more likely than males to report more depressive symptoms and to make greater efforts to seek medical help than males. This greater frequency of treatment-seeking behaviour in females might contribute to a greater likelihood that they receive a diagnosis of depressive disorder. This suggests that female-oriented response bias could possibly be operating in the reporting of symptoms and contributing to the differences observed in certain depressive symptom clusters and the overall prevalence rate disparity between the sexes (Hankin et al., 2008; Nolen-Hoeksema, 1987; Trivers, 1972). Studies have shown that response bias operates in two ways: first, females are considered to show greater endorsement of depression symptoms, and second, males show more reluctance to report such symptoms. These response patterns are thought to be influenced by socialisation processes and gender role expectations, particularly those concerning emotionality (Sigmon et al., 2005). Sex differences in emotional response patterns represent some of the most commonly held stereotypes in Western culture and will be explored here as a means of highlighting the impact that socialisation and gender-expectation

have on reporting behaviour (Barrett, Lane, Sechrest, & Schwartz, 2000; Timmers, Fischer, & Manstead, 2003; Whittle et al., 2011).

In contrast to males, females are often considered to be more emotionally expressive and reactive (Whittle et al., 2011), better at decoding non-verbal emotional cues (Rosip & Hall, 2004; Schulte-Rüther, Markowitsch, Jon Shah, Fink, & Piefke, 2008), more capable of showing greater complexity and differentiation of people's representations of emotional experiences (Barrett et al., 2000), and more accurate than males in identifying others' facial expressions (Thayer & Johnsen, 2000). There is also evidence to suggest that females report emotions with greater intensity than males (Brebner, 2003). In a review of fear and sadness, Madden, Barrett and Pietromonaco (2000) suggested that women were more verbally and non-verbally expressive of fear/sadness than men and reported such emotions with more intensity. Despite findings such as these, some studies have not identified any gender differences in emotional responsivity (Coie & Dodge, 1998). Christov-Moore et al. (2014) argued that the social stereotype of women being more empathetic than men may increase their motivation to appear so when reporting their emotional experiences. Interestingly, Madden et al. (2000) suggested that women who express their emotions are more likely to receive an immediate positive response from others which reinforces gender-based stereotypes of such behaviour.

Sex differences in emotional responding however, have also been identified at the biological level, which may assist in explaining potential underlying influence on culturally-based stereotypes. Neuroimaging studies investigating the neural correlates of emotional processing in typically developing individuals have identified regional differences in brain activation during the completion of tasks designed to tap emotion content. For example, Drobyshevsky, Baumann and Schneider (2006) presented participants with emotionally-charged pictures (e.g., images showing aggressive, erotic, or stressful content) and neutral

pictures (e.g., common objects or smells) whilst measuring brain activation using fMRI techniques. Those researchers reported that females had stronger activation in the occipital cortex while males showed more extensive activation in the frontal cortex, inferior temporal gyrus, middle temporal gyrus, posterior cingulate, and amygdala. Hofer et al. (2007) used positively- and negatively-valenced words to invoke emotional responses in order to examine the brain regions involved in emotional processing using fMRI techniques. For positive stimuli, women showed greater activation in the right putamen, right superior temporal gyrus and left supramarginal gyrus compared to men. For negatively-valenced words, women showed greater activation in the left perirhinal cortex/hippocampus compared to men but men had relatively greater activation in the right supramarginal gyrus compared to women. Despite these biological underpinnings, the evidence for response biases operating in the reporting of depression symptoms remains unclear. A study conducted by Delisle et al. (2012) found no indicators of reporting bias after assessing 470 female patients matched with male counterparts on cognitive/affective symptom scores on the Beck Depression Inventory-II (BDI-II). That research team observed minimally higher somatic symptom scores for women versus men and suggested that differences in the experience and reporting of such symptom would not likely explain gender differences in depression rates and symptom severity.

Gender roles and social identity may impact on disparate prevalence rates due to their association with psychosocial stressors that differentially affect men and women. Masculine and feminine stereotypes coupled with gender-based socialisation processes that are intensified during adolescence may contribute in part to such aspects as symptom reporting patterns, quality and quantity of symptoms and recall bias (Klose & Jacobi, 2004; Kuehner, 2003). In childhood, socialization agents (including parents, peers, teachers and the media) may encourage boys and girls to conform to gender-based expectations of behaviour by differentially reinforcing boys and girls who engage in behaviour considered appropriate for

their sex (Christiansen, 2015; McLean & Anderson, 2009; Madden, Barrett, & Pietromonaco, 2000). In regards to anxiety and fear, Christiansen (2015) suggests that young boys are generally encouraged to confront feared objects, resulting in a greater exposure and extinction of fear responses in males compared to females, for whom avoidance and fear-oriented behaviour are more likely to be tolerated. McLean and Anderson (2009) also suggest that learning to cope with anxiety in a problem focused manner may equip men with the skills that prevent the development of anxiety disorders, while the feminine gender role that emphasises dependency and expectations of protection would be more compatible with avoidance behaviour. Females are also more likely to respond to experiences of stress with rumination, where internalising feelings of distress and personal concerns put them at an increased risk of developing depressive disorders across the lifespan and may also influence the greater frequency with which they report symptoms (Christiansen, 2015; Nolen-Hoeksema, 2001). Associations between poor mental health status and socio-demographic factors have been examined in relation to role conflict and role overload (Nolen-Hoeksema, 2001). However, researchers have failed to find any association between the number or type of social roles held by men and women and the onset of depression and anxiety (Klose & Jacobi, 2004; Weich, Sloggett, & Lewis, 2001). Such results suggest that disparity in prevalence rates for depression and anxiety disorders may instead exist in differential symptom manifestation. It is likely that social and gendered factors alone have minimal impact on depression. Instead their influence might occur via interaction with biological variables, leading to the development of depression and anxiety disorders; with men and women responding in sex- and gender-specific ways to the experience of these disorders (Afifi, 2007; Nazroo, Edwards, & Brown, 1998). Therefore, sex differences in the course of depression and anxiety (as opposed to onset of the disorder) may be responsible for the higher prevalence rates favouring females (Piccinelli & Wilkinson, 2002).

Studies of depression support the view that men and women do exhibit different patterns of depressive symptoms. Women diagnosed with a depressive disorder are more likely to present with symptoms such as increased appetite and weight gain, and report greater anxiety symptoms, psychomotor retardation, hypersomnia, somatic indicators and concerns about body image and physical appearance, whereas men tend to exhibit greater levels of insomnia and weight loss (Johnson, 2001; Kuehner, 2003; Sagud et al., 2002). Crick and Zahn-Waxler (2003) has suggested that symptom profiles in adolescent females also vary from those of their male counterparts with young females reporting more crying, sadness and negative self-concept than young males. Silverstein (1999), analysed interview data on major depression from the National Comorbidity Survey from 8098 men and women aged 15-54 years and concluded that the gender variation in depression may result primarily from a difference in anxious symptoms (such as fatigue, appetite and sleep disturbance). Females in this study exhibited twice the prevalence of somatic depression as did male subjects, whereas the prevalence rates were similar for "pure" depression (described as depression not associated with sleep and appetite disturbance, fatigue, and anxiety). Sex differences have also been noted in treatment settings. In a study that reviewed over 26,000 psychiatric hospitalisations from 1995, Kessing (2005) found that more women than men were treated for depression as outpatients than inpatients and received treatment for longer periods of time in both settings. Further, a greater number of women who were treated as outpatients experienced mild and moderate levels of depression compared to severe depression. No gender differences were found in the inpatient group.

Studies focused on sex-based influences in anxiety have also reported a number of differences that warrant consideration. For example, Bekker and van Mens-Verhulst (2007) reviewed four empirical studies concerning anxiety disorders and the effects of gender on treatment outcome following pharmacological and psychotherapeutic treatment. Those

researchers discovered that, of two studies which assessed the effects of pharmacologic treatment with Sertraline for panic disorder and GAD, only one of these reported that women displayed a greater positive treatment response. Interestingly, use of psychotherapeutic approaches (i.e., Cognitive Behaviour Therapy and Psychodynamic Therapy) with anxiety disorder did not reveal any sex differences in treatment outcomes, although males were more likely to withdraw from therapy after initial intake. Finally, the use of intensive residual therapy with obsessive-compulsive disorder resulted in better outcomes for women who reported a decrease in the symptom of making complaints. Lewinsohn, Gotlib, Lewinsohn, Seely, and Allen (1998) assessed 1,709 adolescents (mean age of 16.6 years) at an initial assessment where participants completed a diagnostic interview and questionnaires designed to assess psychosocial variables associated with depression. One thousand five hundred and seven of these participants were again assessed at a subsequent interview during a return visit approximately 1 year later. That research team reported two significant findings following analysis of the interview data. First, there were no male-female differences in age of onset or duration of the first anxiety episode but the rate at which females developed anxiety disorders was faster than that of males. Second, females reported greater psychosocial impairment than males including significantly more major life events, higher self-consciousness, lower self-esteem, greater emotional reliance, more physical illness and a greater number of physical symptoms as well as rating themselves to have higher social competence.

5.3: Biological Underpinnings of Sex Differences in Depression and Anxiety Disorders

Studies attempting to identify the underlying causes of the sex differences discussed above in Section 5.2, have implicated various biological and psychological factors as contributors (Altemus, 2006; Breslau, Schultz, & Peterson, 1995; Leach et al., 2008; Sagud et al., 2002) and high comorbidity rates between depression and anxiety disorders suggest shared genetic aetiology and biological influences, including genetic determinants, disruption of neurotransmitter systems and fluctuations in reproductive hormones (Altemus, 2006; Breslau et al., 1995; Leach et al., 2008; Nemeth et al., 2013; Nestler et al., 2002).

Twin and family studies indicate genetic factors play an important role in the development of depression and anxiety disorders. The frequent co-occurrence of depression and anxiety suggests there is a set of unified genetic factors at play, with environmental factors (such as peer interactions, specific stressors, or illness) being attributed to variability across symptom profiles (Burton et al., 2015). Overall, twin studies suggest a heritability of 40-50% for MDD and 30-40% for anxiety disorders (Burton et al., 2015; Lohoff, 2010) and family studies indicate up to a threefold increase in lifetime risk of developing MDD or anxiety among first-degree relatives (Burton et al., 2015; Domschke & Dannlowski, 2010; Levinson, 2006). Modest heritability across these disorders suggests that a significant proportion of variance in liability (the underlying risk toward manifestation of a condition, where liability above a certain threshold results in expression of the condition and liability below the threshold results in normality, Hartl, 2014) can be attributed to environmental factors (Hettema, Neale, & Kendler, 2001). That is, although genetic vulnerability is critical to the development of depression and anxiety incidence rates are low in the absence of contributory environmental factors (Young & Korszun, 2010).

Sex differences in genetic liability for depression have been explored in the research with some studies reporting a greater genetic contribution to depression or depressive

symptoms among boys compared to girls, while environmental factors appear to play a more significant role in girls compared to boys (Chen & Yu, 2015; Uddin et al., 2010). Other studies however, have suggested that genetic contributions are more influential in girls (Silberg et al., 1999). Uddin et al. (2010) suggested this greater effect in girls may be due to gene-environment interactions, where the genetic influences on depression become evident only under particular environmental conditions. Research suggests that men and women experience similar rates of stressful life events, a precursor to the onset of depression, but differential sensitivity to such events may instead contribute to the female preponderance in depressive disorder (McEwen, 2005; Piccinelli & Wilkinson, 2002; Stevens & Hamann, 2012; Young & Korszun, 2010). Hankin, Mermelstein and Roesch (2007) for example, found that adolescent girls' greater exposure to interpersonal stressors, particularly those in the family and peer domains, mediated more of the sex differences noted in adolescent depression. Piccinelli and Wilkinson (2000) suggested an increased risk in the onset of depression may be expected when severe events occur in life domains to which individuals attached a strong sense of value. This was supported by a study conducted by Nazroo, Edwards, and Brown (1998) who hypothesised that specific events would have differential effects depending on the salience of those events to the role identities held by the men and women experiencing them. In their study, these researchers reported that women were at a greater risk of developing depression following a crisis that involved children, housing, and reproduction; whereas no differences were found between the sexes when the crisis involved finances, work and marital relationships. Interestingly however, for women who had a greater role salience for children, housing, and reproduction crises, there was a tenfold greater risk of developing depression compared to women who did not have a great role salience for these factors. This implicates gendered factors as additional influences in the development of depression. Causal links between stress exposure and anxiety disorder have also been reported and evidence

suggests that sex differences in anxiety may also correspond with differential genetic susceptibility or related vulnerability factors (Eley, 2007; McLean & Anderson, 2009).

In addition to genetic susceptibility, biological bases for depression and anxiety disorders concern alterations in the mechanisms that underlie the stress response. These disorders share common pathophysiology and differences in the brain (at the anatomical, molecular and cellular levels) can contribute to sex differences in disease vulnerability and severity (Bangasser & Valentino, 2014). Neuroimaging studies of depressed patients have identified several abnormalities of regional cerebral blood flow and glucose metabolism (which provides the energy necessary for physiological brain function including such processes as neuronal and non-neuronal cellular maintenance, the generation of neurotransmitters, and neuronal computation and information processing, Campbell, Muncer, & Coyle, 1992) in various brain regions, including the limbic cortex, the prefrontal cortex, the hippocampus, the amygdala, striatum, thalamus, and the anterior cingulated cortex (Kalia, 2005). Knowledge of the function of such brain regions in typically developing individuals provides insight into the aspects of depression they meditate (Nestler et al., 2002). These brain regions are responsible for governing various cognitive abilities and behaviours (including executive functions, understanding and moderating social behaviour, and emotion regulation) and abnormal functioning in these areas may contribute to difficulty mediating the symptoms characteristic of depression and anxiety disorders (Martin, Ressler, Binder, & Nemeroff, 2009; Nestler et al., 2002). Nestler et al., (2002) suggested that the neocortex and hippocampus may mediate the cognitive aspects of depression, including memory impairments and feelings of worthlessness, hopelessness, and suicidality, while the striatum and amygdala (implicated in emotional memory) may mediate aspects such as anhedonia, anxiety, and reduced motivation. Across the different subtypes of anxiety, neuroimaging studies have consistently implicated increased activation of the amygdala, which is involved

in several fear-related processes such as fear conditioning (Duvarci, Popa, & Paré, 2011; Etkin & Wager, 2007). Fear conditioning is a form of associative learning whereby a neutral stimulus (conditioned stimulus, CS) is paired with an aversive stimulus (unconditioned stimulus, US) resulting in the CS acquiring the aversive properties of the US. When presented in the absence of the US, the CS will elicit responses characteristic of fear, including increased autonomic nervous system activity and behavioural reactions such as freezing (Campbell et al., 1997). Other brain regions implicated in anxiety disorders include the insula, which is responsible for emotion processing as well as subjective feelings and interoceptive awareness, the anterior cingulate cortex, affiliated with avoidance and fear learning, the hippocampus, which plays a role in mediating emotional responses to the context of a stressor, the prefrontal and parietal cortex, associated with the cognitive aspects of the stress response through its role in placing a threatening object in space and time, and finally the neocortex, which is involved in the preparing for a response to a threat (Bremner & Charney, 2010; Holzschneider & Mulert, 2011).

The interactional nature in which these brain regions operate also allows for the identification of neural circuitry and neurochemical mechanisms that may underlie depression and anxiety disorders (Young & Altemus, 2004). The HPA axis, a major mechanism involved in the stress response, plays a key role in explaining the biological underpinnings of depression and anxiety. The HPA axis functions via a negative feedback loop that regulates the release of glucocorticoids, which prepare an individual to adapt to and cope with stressors that affect behaviour by acting on various brain regions and other parts of the body involved in maintaining a homeostatic state (Freidenberg et al., 2010; Nestler et al., 2002; Swaab, Bao, & Lucassen, 2005). More specifically, the release of corticotropin releasing factor (CRF) and vasopressin from the paraventricular nucleus of the hypothalamus (PVN) stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the blood. Circulating

ACTH then acts on the cortex of the adrenal glands to induce the production of glucocorticoids (cortisol in humans), that then feedback on glucocorticoid receptors (GR) in the PVN and pituitary to supress activation of this system (Bangasser & Valentino, 2014; Freidenberg et al., 2010; Young & Altemus, 2004). GR receptors are also found in various other brain regions involved in cognitive function, including the hippocampus, amygdala and prefrontal cortex (Swaab et al., 2005). Appropriate glucocorticoid negative feedback is important because, despite the metabolic and immune changes induced by glucocorticoid release being beneficial in the short term, their maintenance over longer periods causes detrimental effects to health (Bangasser & Valentino, 2014; Freidenberg et al., 2010). Abnormal and excessive activation of the HPA axis has been observed in male and female patients with depression and anxiety disorders and has been implicated as a contributory factor in the sex-based differences observed between men and women diagnosed with these disorders (Nemeth et al., 2013). Studies of depression for example have shown depressed women to have higher cortisol levels compared with their male counterparts (Bangasser & Valentino, 2014; Piccinelli & Wilkinson, 2002). In a study of 87 inpatients (43 males and 44 females) conducted by Matsuzaka et al. (2013) female patients were found to have significantly increased levels of serum cortisol compared to female controls, while no significant differences were reported between male patients and male controls. Young and Korszun (2010) assessed plasma cortisol concentration in 16 depressed patients matched for age and sex with 16 healthy controls and reported female patients as having significantly higher cortisol levels than their matched controls, while male patients and their matched controls showed the same plasma cortisol concentrations. Studies of PTSD suggest that women experience lower levels of cortisol than men. In an assessment of 3 men and 6 women with PTSD, Freidenberg et al. (2010) found that women had lower basal cortisol levels than men, while men had greater

cortisol concentrations than are typical, indicating that glucocorticoid secretion is disrupted in a sex-specific manner among men and women with PTSD.

Such results suggest that sex differences in cortisol levels between male and female patients diagnosed with an anxiety disorder, stem from sexually dimorphic HPA axis activation in response to stressors. Conflicting results reported in other studies suggest that sex differences in the magnitude of the stress response are insufficient in explaining the increased sensitivity of women to psychiatric disorders related to this response (Hinkelmann et al., 2012; Young & Korszun, 2010). Greater HPA axis reactivity in females compared with males may be influenced by variations in oestradiol levels that occur across the oestrous cycle (Piccinelli & Wilkinson, 2002). Changes in brain structure brought about by the organisational effects of hormones produced at puberty, during pregnancy, and at menopause, coupled with the activational effects of circulating hormones across the oestrous cycle are also likely to influence the development of affective disorders in women compared to men (Altemus, 2006; Swaab et al., 2005; Young & Korszun, 2010). Altemus (2006) suggested that increased responsivity of the HPA axis, coupled with a decrease in glucocorticoid feedback sensitivity and decreases in brain GABA content during the luteal phase of the menstrual cycle, potentially disrupt the stabilising feedback systems in women vulnerable to developing an affective disorder (Altemus, 2006). Swaab et al. (2005) suggested that further evidence for the influence of gonadal hormones on the development of these disorders comes from the interaction of the HPA and HPG axes. In depressed women, dysregulation of the HPG axis and increased activity in the HPA axis results in decreased plasma levels of oestrogen and an increase in testosterone levels respectively, as testosterone in women is regulated in the adrenal glands. Dysregulation of the HPG axis in men, however, results in decreased levels of testosterone, given that this hormone in men is produced in the testis (Altemus, 2006; Swaab et al., 2005). Further research is needed however, to understand the manner in which gonadal

steroid hormones influence HPA function and contribute to sex-based differences in mental disorder (Handa & Weiser, 2014).

Other major neurochemical mechanisms implicated in the development and course of depression and anxiety disorders include the serotonergic system (associated neurotransmitter: serotonin), dopaminergic system (associated neurotransmitter: dopamine), benzodiazepine system (associated neurotransmitter: gamma-Aminobutyric acid, or GABA) and the noradrenergic system (associated neurotransmitter: norepinephrine) (Bremner & Charney, 2010; Möhler, 2012). Each system is involved in the regulation of various behavioural states through the release of neurotransmitters that act on various brain structures to effect physiological and behavioural change. Low levels of serotonin have been implicated in poor impulse control, sleep irregularities and bias the brain toward negative appraisal and pessimism; diminished dopamine levels are associated with loss of pleasure and reward and insufficient attention; reduced GABA is connected with feelings of agitation; and finally, imbalances of norepinephrine are linked to high arousal and agitation (Bremner & Charney, 2010; Burijon, 2007; Thase, 2009; Wehrenberg, 2014). A thorough exploration of each system is beyond the scope of this thesis, and the present discussion will instead present an overview of the serotonergic system, which represents the most researched system in relation to mood and anxiety.

Serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter synthesised in the brain by distinct cell clusters in the raphe nuclei in the brain stem. Caudal neurons extending to the cerebral cortex, hypothalamus, thalamus, basal ganglia, septum, and hippocampus have been implicated in depression, while serotonergic pathways located in the amygdala, rectus gyrus of the frontal lobe, and the inferior and superior gyri of the temporal lobe have been associated with anxiety disorders (Burijon, 2007; Thase, 2009). 5-HT functions to coordinate complex sensory and motor patterns during a variety of behavioural states, including mood,

impulse control, sleep, vigilance, eating, libido, and cognitive functions (such as memory and learning), as well the modulation of anxiety and fear (Akimova, Lanzenberger, & Kasper, 2009; Cosgrove et al., 2007; Weiss, Abney, Cook, & Ober, 2005). 5-HT neurons express membrane-bound transporters (5-HTT), which synaptic transmission. It has been suggests that reduces availability of 5-HT may be associated with depression (Thase, 2009). Selective serotonin reuptake inhibitors (SSRIs), which are commonly used in the treatment of clinical depression and anxiety, act by selectively blocking the reuptake of 5-HT following its release from neurons, thereby increasing the available 5-HT, which leads to improved 5-HT neurotransmission in the brain (Akimova et al., 2009; Drevets et al., 2007). Reduced 5-HT neurotransmission is considered to result in an enhanced response to stress and emotional disturbance (Mizuno et al., 2006). The 5-HT1A receptor has also been implicated as particularly influential in depression and anxiety disorders due to its presence in several cortical and subcortical areas and is considered as a major inhibitory serotonergic receptor (Akimova et al., 2009). Activation of presynaptic 5-HT1A receptors by 5-HT results in a reduction of the firing rate of these neurons, the amount of serotonin released per action potential, and the synthesis of the neurotransmitter, and thus the associated serotonergic activity in other brain areas to which the system extends, while activation of postsynaptic 5-HT1A receptors modulate serotonergic sensitivity and are involved in emotional and cognitive processes (Akimova et al., 2009; Savitz, Lucki, & Drevets, 2009). Reduced regulation of 5-HT1A receptors is a consequence of exposure to chronic stress and dysregulation of these receptors contributes to symptoms of depression and anxiety (Burijon, 2007; Thase, 2009).

A number of sex differences have been reported in relation to 5-HT function. Ortiz, Artigas, and Gelpí (1988) assessed plasma and whole blood 5-HT, as well as its plasma-bound metabolite 5-HTAA in 175 individuals (83 males and 92 females), finding women to have higher levels of both, which may reflect a differential whole body 5-HT function. Nishizawa et

al. (1997) measured the rates of serotonin synthesis in the brains of 14 individuals (8 males and 7 females) using PET imaging and reported that 5-HT syntheses occurred at a greater rate (52%) in males compared to females. Jovanovic et al. (2008) studied sex differences in 5-HT1A receptor and 5-HTT binding potentials in the brains of 14 men and 14 women via PET imaging. Women had a 39% higher 5-HT1A receptor binding potential compared to men, while men had a 55% higher 5-HTT binding potential compared to women. Sexual dimorphism in 5-HT function may underlie the disparate prevalence rates observed in depression and anxiety across genders (Jovanovic et al., 2008; Staley et al., 2005). In a study of 32 depressed patients matched for age, sex and smoking status with 32 healthy subjects, Staley et al. (2005) found the 5-HTT availability in the diencephalon was lower in depressed women in an age-dependent manner but not in depressed men, suggesting that, due to higher baseline levels of 5-HT function in women versus men, dysregulation of this function in depressed women may contribute to sex-specific mechanisms underlying depressed mood in women.

Serotonin levels are also impacted upon at a genetic level, and may be an influential factor in differing male and female susceptibility to anxiety and depression. Weiss, Abney, Cook, and Ober (2005) conducted a genome-wide linkage and association study that aimed to identify sex differences in the genetic contribution to differential levels of serotonin between males and females. They measured whole blood serotonin in 567 individuals (300 females and 267 males) and reported sex-based differences in both whole blood serotonin levels and in the underlying genetic architecture. Specifically, female heritability was shown to have a larger additive component and different loci influenced serotonin levels in a sex-specific manner. Quantitative trait loci (QLTs; genetic loci that contribute to variations in quantitative phenotypes, Wang, Barratt, Clayton, & Todd, 2005) on chromosomes 17q and 2q are specific to males, whereas a locus on chromosome 6q is specific to females, demonstrating that the

loci influencing whole blood serotonin levels differ between males and females. Heritability is also partly determined by polymorphism long (*l*) and short (*s*) alleles in the linked promoter region of the 5-HTT gene (5-HTLPR). Variation in this gene has been associated with sensitivity to stress and sex has also been implicated as a mediating factor in the differential expression of 5-HTTLPR (Mizuno et al., 2006; Thase, 2009). Mizuno et al. (2006) assessed sex-related variation in the 5-HTTLPR with different levels of stress perception, anxiety and depression in 104 patients (45 males and 59 females) and 90 controls (53 males and 37 females) reporting that females with the *l*/*s* genotype showed higher scores on all emotional measures in comparison to males with the same genotype. No sex differences were reported for the *s*/*s* genotype and the *l*/*l* genotype was excluded due to insufficient numbers. These results led Mizuno et al. (2006) to suggest that 5-HTTLPR *l* allele may be related to negative emotion in females, but that it may act differently in males.

In addition to differential genetic effects on serotoninergic function, animal studies have implicated gonadal sex hormones as possible mediators in serotonin system functioning. The presence of estradiol-17β in female rats increases the amount of 5-HT2A receptor mRNA in the dorsal raphe nucleus with an associated increase in the density of 5-HT2A receptor binding sites in the frontal, cingulate and primary olfactory cortex, the nucleus accumbens and caudate-putamen, brain regions, which have been implicated as mediators of mood, mental state and cognition (Fink, Sumner, Rosie, Wilson, & McQueen, 1999). In male rats, testosterone has been shown to regulate 5-HT1A and 5-HT2A receptor mRNA levels in a region-specific manner. Gonadectomised male rats, compared to controls, demonstrated significant increases in cellular 5-HT1A 5-HT2A mRNA levels in the cortex, hippocampus, hypothalamus and amygdala, where the androgen receptors are abundant, as well as in the dorsal raphe nucleus, suggesting that testosterone differentially regulates 5-HT receptor subtype expression in rats (Zhang, Ma, Barker, & Rubinow, 1999). Findings such as these

implicate gonadal steroids as influential in the regulation and development of the serotonergic system whereby sexually dimorphic brain circuitry then mediates sex-specific physiology and behaviour.

Chapter Six: Sex Differences in Autism Spectrum Disorder

Evidence demonstrating the occurrence of sex-based variation in mental disorder (as explored in the previous discussion, that was focused on depression and anxiety), also raises questions as to whether or not sex-based differences are also present in neurodevelopmental disorders. This is particularly relevant to the context of those early-onset childhood disorders in which marked male preponderance has been consistently reported. It may be that this over-representation of males reflects an underlying sex-based variation.

The term neurodevelopmental disorders refer to a group of conditions associated with impairments in the growth and development of the brain or the central nervous system which then impedes brain function and behaviour. These disorders become evident early in development by 36 months of age, and are usually manifest in relation to delayed development in age-appropriate skills and presence of atypical behaviours which undermine functioning (APA, 2013; Van Herwegen, Riby, & Farran, 2015). This constellation of developmental impairments, whilst capable of some remediation and improvement, pervades various life areas and across the lifespan (Bölte, Duketis, Poustka, & Holtmann, 2011). Establishing the impact of sex-based variation in neurodevelopmental disorders has important implications for understanding their clinical presentation and developmental trajectory, particularly when early detection and intervention is a key factor in establishing positive outcomes later in life (Lord, Luyster, Guthrie, & Pickles, 2012).

6.1: Overview of Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is the most recognisable and well-researched neurodevelopmental disorder and, in Australia and other western countries such as the USA, is detected with reference to the diagnostic criteria set out in the Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition (DSM-5, APA, 2013). The DSM-5 uses the term ASD to represent a spectrum of related conditions which, whilst they might vary in symptom expression and severity, are all characterised by pervasive impairments in social communication and reciprocal social interaction (including deficits in social-emotional reciprocity, nonverbal communicative behaviours required for social interaction, and developing, understanding and maintaining relationships) and the presence of repetitive and restricted behaviour, activities and interests (RRBI's) (including stereotyped or repetitive motor mannerisms, an insistence of sameness, preoccupation with specific topics, and unusual reactions to or interest in sensation) (APA, 2013). These impairments cause delay in the normal development and progression of adaptive behaviours and skills that are fundamental to optimal functioning across important domains including social, psychological, academic, and educational/occupational (Baghdadli, 2012; Feinstein, 2011). Moreover, the onset of symptoms must occur prior to 36 months of age in order to meet the diagnosis of ASD. Signs of social and communication impairment (such as maintaining eye contact, engaging in pretend play, and showing appropriate interest in object and/or people) may be present as early as 12 months for severely affected children, whereas, high-functioning individuals might not show clear evidence of disorder until they confront the more complex social demands of school (Robins, Fein, Barton, & Green, 2001**;** Pandey et al., 2008; Shumway et al., 2011; Szatmari, 2011).

As implied by the term 'spectrum', the degree to which individuals with ASD experience impairment varies widely. The condition itself is highly heterogeneous, and

clinical features may be further exacerbated by the presence of additional difficulties. That is, varying symptom severities and the occurrence of comorbid psychopathology and/or associated conditions impact an individual's functioning across key developmental areas including social/emotional (e.g., friendships, interaction, working cooperatively), language/communication (e.g., receptive and expressive language), motor/physical (e.g., fine and gross motor skills), cognitive (e.g., spatial reasoning and processing speed), and behavioural (e.g., aberrant behaviours that although not specific to autism may impact functioning in other domains) areas (Saulnier & Ventola, 2012). In relation to social/emotional functioning, some individuals with ASD may avoid social interaction altogether, even when another person initiates it, while other individuals may actively seek interaction, although in an unusual manner (Geschwind & Levitt, 2006; Scheeren, Koot, & Begeer, 2012). Moreover, not all individuals demonstrate repetitive sensory motor mannerisms (such as hand flapping or rocking) or an insistence on sameness (e.g., resisting changes in environment and routines) (Cuccaro et al., 2003).

'Comorbidity' refers to the presence of two or more disorders in the same person (Matson & Nebel-Schwalm, 2005). The types of variation (and the most commonly-occurring conditions that fall within these categories) which impact the clinical features of ASD include cognitive (Intellectual Impairment, Joseph, Tager–Flusberg, & Lord, 2002), psychiatric (mood disturbance, Brereton, Tonge, & Einfeld, 2006; anxiety and phobias, Brereton et al., 2006; Hayashida, Anderson, Paparella, Freeman, & Forness, 2010), and behavioural (Obsessive-Compulsive Disorder [OCD], Gadow, DeVincent, Pomeroy, & Azizian, 2004a; Hayashida et al., 2010; Attention Deficit and Hyperactivity Disorder [ADHD], Brereton et al., 2006; Gadow et al., 2004a; Tourette's disorder, Gadow et al., 2004a; and sleep problems, Tudor, Hoffman, & Sweeney, 2012) conditions. When present, these comorbidities often require additional clinical attention to the development and implementation of interventions that aim to reduce

overall impairment and improve quality of life (Simonoff et al., 2008). Similarly, the presence of associated difficulties, or those additional features/behaviours that are not required for a diagnosis of autism, may also impact upon the manner in which an individual responds to his/her environment. Research (e.g., Matson & Nebel-Schwalm, 2005; Simonoff et al., 2008) suggests that individuals with ASD are highly likely to develop atypical or challenging behaviours, which are often intense and disruptive, causing harm to the individual and others. These behaviours, which result from impairment in sensory, motor skill, cognitive and social domains (Jansiewicz et al., 2006; Nadon, Feldman, Dunn, & Gisel, 2011; Robertson & Simmons, 2013) include aggression, self-injury, property destruction, noncompliance and stereotypies (Matson, Neal, Fodstad, & Hess, 2010). Here, the term 'associated difficulties' used to distinguish between those symptoms of a diagnosed comorbid disorder and the presence of additional behaviours that do not constitute a comorbid diagnosis.

In recent decades, global prevalence rates for ASD (including the previously defined diagnostic labels of Autistic Disorder [AD], Asperger's Disorder [AS] and Pervasive Developmental Disorder – Not Otherwise Specified [PDD-NOS]) have been steadily increasing (Roth, 2013; Williams, MacDermott, Ridley, Glasson, & Wray, 2008). In Australia, the 2005/2007 national prevalence estimates indicated a rate of 1 in 160 children aged between 6 and 12 to be diagnosed with ASD (Autism Advisory Board, 2011**;** Buckley, 2013). By 2009, this rate had increased to approximately 1 in 90 for the same age group (Buckley, 2013). More recently however, prevalence was estimated to be 1 in 61.5 for children of school age, based on data obtained from the 2012 Survey of Disability, Ageing and Carers (SDAC) (Australian Bureau of Statistics [ABS], 2012; Buckley, 2013). Other countries have reported similar prevalence rates. For example, the American Centers for Disease Control and Prevention (CDC) Autism and Developmental Disabilities Monitoring (ADDM) Network estimated the prevalence of Autism in 2012 to be 14.6%, affecting 1 in 68 children aged 8 years (Centers for

Disease Control and Prevention, 2016). A number of explanations for the rise in ASD prevalence have been proposed. Prevalence estimates may have been influenced by changes in diagnostic criteria, increases in awareness and understanding, ascertainment of data from different sources/databases, the use of different assessment instruments and research methodologies to collect data, and cultural differences (Baird et al., 2006; Matson & Kozlowski, 2011; Roth, 2013; Van Herwegen et al., 2015).

Research concerning the aetiology of ASD suggests that this group of conditions may result from a complex interaction between biological and environmental factors, as no single genetic or neuroanatomical factor has been identified that can solely account for to the development of ASD (Gupta, & State, 2007; Ratajczak, 2011). At the genetic level, variation is thought to influence the presentation of symptoms, as evidenced by the phenotypic diversity observed across the ASD population, as well as susceptibility to the condition (Abrahams, 2011). Evidence of a genetic predisposition has been derived from twin and family studies, which have shown an increased risk for an individual to be diagnosed with autism if a sibling has received an ASD diagnosis (Abrahams, 2011; Lauritsen, Pedersen, & Mortensen, 2005). Recent studies investigating the heritability of autism in twins have yielded consistent results and indicate strong concordance among monozygotic (MZ) twins, who are genetically identical, and dizygotic (DZ) twins, who share approximately 50% of their genes. Using the Childhood Autism Rating Scale (CARS), administered via interview and/or direct observation to measure presence and severity of ASD symptoms (Chlebowski, Green, Barton, & Fein, 2010), Taniai et al. (2008) assessed the severity of autistic traits in 45 twin pairs recruited from a screening program aimed at identifying developmental problems in children from 3 months of age. The study sample was comprised of 19 MZ pairs and 26 DZ pairs and reported concordance rates of 94% and 30%, respectively. Similar results were reported by Rosenberg et al. (2009), who conducted a study of 277 twin pairs recruited from a voluntary online

research database, the Interactive Autism Network. Researchers utilised two screening tools to assess autism symptoms; the Social Responsiveness Scale (SRS), which provides a quantitative measure of autistic traits in children aged from 4 to 18 years old (Boltë, Poustka, & Constantino, 2008) and the Social Communication Questionnaire (SCQ), which is used to identify individuals at risk of developmental problems (Allen, Silove, Williams, & Hutchins, 2007). Concordance rates were reported at 88% in MZ twin pairs and 30% in DZ twin pairs. In their population-based study of 117 twin pairs identified in the Child and Adolescent Twin Study in Sweden, Lichtenstein et al. (2010) estimated overall heritability of ASD to be 80%. Participants in this study were assessed using the Autism–Tics, ADHD, and Other Comorbidities inventory (A-TAC), a parent-report screening tool developed to identify a number of neurodevelopmental disorders including ASD (Larson et al., 2010). In addition, candidate gene studies, which assess the potential contribution of specific genes to ASD susceptibility based on the gene's function, have suggested possible links to numerous chromosomes with loci identified on 1p, 2q, 3p, 7q, 15q, 17q and the sex chromosomes showing the strongest evidence (Auranen et al., 2002; Chung et al., 2011; Esser et al., 2010; Jamain et al., 2003; Larson et al., 2010; Liu et al., 2011). Identifying genes linked to ASD may assist in the identification of pathways and systems important in understanding the development of this condition (Larson et al., 2010).

Neuroimaging, techniques have also implicated abnormalities in brain structure in the development of autism (Herbert, 2011). While such studies have played a major role in advancing understanding of the neural underpinnings of ASD, there is considerable heterogeneity among these findings, making the identification of ASD-specific neural patterns difficult (Anagnostou & Taylor, 2011; Mazzone & Curatolo, 2010). Nevertheless, neuroimaging techniques have been used to support the relationship between abnormal brain structure/function and the clinical features of ASD. In a review of fMRI and DTI studies

conducted between 1990 and 2011, Pina-Camacho et al. (2012) identified 120 original investigations that used these techniques and described abnormalities in brain structure and connectivity in participants diagnosed with ASD in comparison to control groups. For instance, those authors found that impairments in the cortical and subcortical regions and networks such as the prefrontal cortex, inferior frontal gyrus, temporal and cingulate cortex, or the amygdala-fusiform system were associated with the social deficits experienced by individuals on the autism spectrum.

Another area of imaging research that is advancing understanding of the ASD brain is resting state electroencephalography (resting-state EEG). EEG measures neurophysiological changes in the cortex and is used to evaluate brain activity (i.e., function) in the absence of stimulation (i.e., task-based performance or sensory stimulation) (Wang, Barstein, Ethridge, Mosconi, Takarae, Sweeny, 2013). EEG has advantages over other methods of neuroimaging as it is less intrusive, can be used across a wider range of developmental periods, is more clinically available and has a higher tolerance for movement (Wang et al., 2013). Further, other forms of neuroimaging typically measure task-induced changes in cortical activity. Making interpretations about the functional implications of such changes is limited in the absence of functional data collected at rest, while EEG data provide this comparison resting-state information (Wang et al., 2013). Coben, Clarke, Hudspeth and Barry (2008) used EEG techniques to study 20 subjects (14 males and 6 females) diagnosed with ASD aged between 6 and 11 years and matched for age, sex and IQ with 20 control subjects. This research team reported that the ASD group, in comparison to controls, showed dysfunctional integration in the frontal and posterior brain regions as well as atypical brain connectivity, indicative of a pattern of underconnectivity.

As a unifying causal biological predictor for ASD has not yet been identified through genetic and neuroanatomical research, other environmental factors have been implicated as

potential contributors to the development and epidemiology of ASD (Daniels, 2006; Ratajczak, 2011). More specifically, environmental agents are hypothesised to influence the risk of developing autism or autism-like behaviours in those people who are genetically susceptible to the condition. Environmental factors that have been implicated include: complications during pregnancy (Ratajczak, 2011); exposure to infections (Libbey, Sweeten, McMahon, & Fujinami, 2005) and exposure to chemicals/toxins, such as arsenic, lead, manganese, pesticides (Dietert, Dietert, & DeWitt, 2011); pharmacological entities, such as thalidomide or valproic acid (although the nature of such pharmacological influences is debated) (Miyazaki, Narita, & Narita, 2005). The influence of various environmental agents has also been suggested due to the heterogenic diversity of symptoms across the spectrum. While such hypotheses support the notion of a multifactorial process of development, research findings on environmental contributors do not provide a sufficient account of, or unified model for, explaining the development of ASD.

6.2: Sex Differences in Autism Spectrum Disorder

6.2.1: Methodological Issues Impacting the Identification of Sex-Differences in ASD.

Methodological differences between studies have often led researchers to propose a female-specific profile for ASD from their own findings or to make broad inferences based on findings from other studies that differentiate between male versus female profiles, without necessarily providing evidence for consistently-present behavioural variations across the sexes (Koenig & Tsatsanis, 2005; Mandy et al., 2012; Rivet, & Matson, 2011a). The issue of whether sex-based phenotypic differences actually exist in ASD is futher confounded by a number of methodological limitations that possibly undermine the veracity of findings reported in this field. Those limitations require exploration in order to clarify the presence of female-specific ASD symptoms and the implications for effective diagnosis and treatment of female-specific ASD profiles.

Table A1 (see Appendix 1, pages 214-224) provides an overview of 30 studies into the female-male variation in ASD profile that were conducted between the years of 1993 and 2015. A literature search of the Ebscohost, Science Direct, Academic Onefile, NCBI and Google Scholar databases was conducted using combinations of the search terms: (i) Autism, Asperger's, ASD, or Autism Spectrum Disorders; (ii) sex/gender differences, or female; (iii) core symptoms, behaviour, profiles. In addition, relevant studies referenced in the initial search results were also included. This search identified 867 studies, which were reduced to only those that reported on phenotypic differences in ASD symptoms/behaviour between males and females. Meta-analytic and review studies were excluded from this analysis but were used to source additional relevant studies. These 30 studies were then examined for their (i) samples and sampling methods, and (ii) data collection methods. These limitations

were explored because they contribute to the selection of participant groups that form the basis of comparision between males and female with ASD.

Factors related to sampling and sampling methods are those that determine the characteristics that guide which participants will be included in or excluded from a study. Inter-study sampling inconsistencies include differences in participants' ages, variability of diagnosis, matching strategies, sample sizes, case ascertainment and confirmation of diagnosis. The review presented in this section of the thesis is based on 30 studies and specifically addresses the methodological limitations believed to create inter-study inconsistencies leading to a poor basis for development of a unified model for ASD presentation in males versus females. Each of these sampling features and their potential for creating inconsistencies in the ASD research are discussed below.

The inclusion of participants of varying ages may make comparisons within and between male/female groups difficult as differing developmental profiles may impact the manner in which symptoms present and the severity to which they are experienced (Mandy et al., 2012). Studies that have implemented a narrow age inclusion criterion are more capable of identifying sex differences related to gender as opposed to developmental level (Sipes, Matson, Worley, & Kozlowski, 2011). Studies included in this review have been organised according to the age ranges employed within each study in order to identify the most common ages represented in the research (see Appendix 2 on page 225 for an overview of these age ranges). The majority of these studies were focused on toddlers (age ranges 2 – 4 years) and children (age ranges 4 to 11 years), while considerably fewer studies were specific to adolescents (age range 11 – 18 years) and adults (age range 18 and above). Of the 30 studies reviewed for this analysis, 23 had limited their samples to particular age ranges/developmental periods, with 8 studies focused on toddlers aged approximatetly between 1.4 and 5.4 years (Andersson, Gillberg, & Miniscalco, 2013; Carter et al., 2007;

Harrop et al., 2015; Hartley & Sikora, 2009; Postorino et al., 2015; Reinhardt, Wetherby, Schatschneider, & Lord, 2015; Rivet & Matson, 2011b [Study 1]; Sipes et al., 2011), 9 on children aged approximatetly between 4 and 14 years (Amr, Raddad, El-Mehesh, Mahmoudc, & El-Gilany, 2011; Baghdadli, Pascal, Grisi, & Aussilloux, 2003; Coffman, Anderson, Naples, & McPartland, 2015; Dworzynski et al., 2012; Gadow, DeVincent, Pomeroy, & Azizian, 2004a ; Koyama, Kamino, Inada, & Kurita, 2009; Kumazaki et al., 2015; Mandy et al., 2012; Solomon, Miller, Taylor, Hinshaw, & Carter, 2012), two on adolescents aged approximatetly between 10 and 16 years (Bölte et al., 2011, Head, McGillivray, & Stokes, 2014), and three on adults (Hattier, Matson, Tureck, & Horovitz, 2011; Lai et al., 2011, Rivet & Matson, 2011b [Study 3]). Only eight studies (Frazier, Georgiades, Bishop, & Hardan, 2014; Hess, Matson, & Dixon, 2010; Holtmann, Bölte, & Poustka, 2007; Kopp & Gillberg, 2011; Kozlowski, Matson, & Rieske, 2012; McLennan, Lord, & Schopler, 1993; Park et al., 2012; Rivet & Matson, 2011b [Study 2]) included participants across age groups/developmental periods. Given the impact that developmental profiles are likely to have on autism symptoms, this lack of studies addressing adolescents and adults with ASD suggests that further research is required to better understand how sex affects autism symptoms at different developmental periods, such as puberty and transitioning into adulthood.

The inter-study limitation relating to variability of diagnosis arises (in part) from the evolution of the ASD diagnosis and how that evolution is distilled into diagnostic criteria. For instance, previous versions of the DSM (e.g., DSM-IV and DSM-IV-TR) conceptualised autism as three unique disorders under the umbrella term of Pervasive Developmental Disorder (PDD). That is, the DSM-IV and DSM-IV-TR did not officially refer to ASD as a diagnostic label. The PDD umbrella encompassed Autism, Asperger's Syndrome, Rett's Disorder, Childhood Disintegrative Disorder and PDD-NOS. In the mid-1990's, clinician researchers such as Lorna Wing began to use the term ASD to refer to Autism, Asperger's Syndrome, and PDD-NOS

(Volkmar & Klin, 2005). Within this DSM-based multi-categorical system of diagnosis, qualitative variations in level of functioning for each diagnostic group (e.g., Autism versus Asperger's) were empirically recognised (De Los Reyes, Thomas, Goodman, & Kundey, 2013; Mandy et al., 2012). Despite this, researchers continued to raise concerns regarding the applicability of the categorical model due to ambiguity in the boundaries of each possible diagnosis. That is, while the diagnosis for Autism was clearly defined, the criteria for Asperger's Syndrome and PDD-NOS were less so, making them difficult to apply in a clinical setting (Kulage, Smaldone, & Cohn, 2014). For instance, the term high-functioning Autism (HFA) has been used interchangeably with Asperger's Syndrome, possibly due to the poor differentiation in diagnostic criteria between these two conditions. Further, PDD-NOS was considered to be problematic because its identification was based on sub-threshold symptomology rather than the presence of clearly differentiated impairments (Kulage et al., 2014; Walker et al., 2004). Diagnostic inconsistencies such as these possibly produced complications in the interpretation of results and heterogeneity within (same study) and between (different studies) participant groups (Carter et al., 2007; Wing & Potter, 2002).

While the latest edition of the DSM (fifth edition) replaces the previous categorical model with a single diagnostic dimension, the majority of studies that addressed sex-based differences in ASD phenotype have been based on previously-defined diagnostic criteria that were described above and which were derived from the DSM-III, DSM-IV, DSM-IV-TR, and/or the ICD-10 to confirm ASD symptoms. Of the 30 studies, 24 reported applying specific diagnostic criteria (Amr et al., 2011; Andersson et al., 2013; Baghdadli et al., 2003; Bölte et al., 2011; Coffman et al., 2015; Frazier et al., 2014; Hattier et al., 2011; Hartley & Sikora, 2009; Hess et al., 2010; Holtmann et al., 2007; Gadow et al., 2004a; Kopp & Gillberg, 2011; Koyama et al., 2009; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012; McLennan et al., 1993; Park et al., 2012; Postorino et al., 2015; Rivet & Matson, 2011b [Study 1]; Rivet & Matson,

2011b [Study 2]; Rivet & Matson, 2011b [Study 3]; Sipes et al., 2011; Solomon et al., 2012), 5 studies did not report any direct application of diagnostic criteria, but confirmed ASD presence using assessment measures that reliably correlated with DSM/ICD criteria (Carter et al., 2007; Dworzynski et al., 2012; Harrop et al., 2015; Kozlowski et al., 2012; Reinhardt et al., 2015), and 1 study did neither (Head, 2014). Research samples consisted of participants with various diagnoses and included combinations of high-functioning autism, Asperger's Syndrome, Autistic Disorder, Autism, Atypical Autism, and PDD-NOS. This high variability of diagnosis across these studies raises concerns regarding the reliability and generalizability of results to possible broader autism phenotypes specific to males versus females on the autism spectrum.

Matching strategies aim to control for a participant feature and/or domain of functioning to determine if the consequences of that feature or domain are specific to the condition, the most common of which include age, sex and cognitive ability (Carter et al., 2007; Jarrold & Brock, 2004; Mandy et al., 2012). Matching for age reduces any developmental effects that may obscure results, whereas matching for sex promotes the creation of equal sex-based sample sizes that facilitate appropriate comparisons to be made between the sexes. Cognitive ability has also been shown to have a moderating effect on autism symptoms, where an increase in symptom severity is associated with a decrease in IQ, particularly in relation to the DSM-5 criterion 2 (i.e., restricted, repetitive patterns of behaviour, interests, or activities) impairments. Further, across the IQ distribution for example, the male to female ratio is approximately 2:1 in children with low intelligence, yet this proportion increases to a ratio of 8-10:1 in children with average or above-average intelligence (Dworzynski, Ronald, Bolton, & Happé, 2012; Frazier, Georgiades, Bishop, & Hardan, 2014; Mandy et al., 2012). Due to greater overall difficulty, females with cognitive impairment are more likely to meet the diagnostic criteria for ASD compared to those without this comorbidity. This is because individuals

without cognitive impairment may not meet clinical cut-offs to be recognised as having ASD despite their having similar results on measures that assess ASD symptoms (Dworzynski et al., 2012). Including factors such as IQ and age as a covariate allows for the evaluation of sex differences in ASD symptomology as well as how such variables may influence symptom variation (Frazier et al., 2014). Matching participants on features such as age and IQ not only facilitates examination of symptom severity and functional level but has the potential to illuminate any sex-based variations in phenotype.

The majority of studies in the current review made attempts to match participants according to a number of features, which included: (i) age; (ii) sex/gender; (iii) cognitive ability (i.e., IQ and DQ); (iv) autism diagnosis; and (v) symptom severity (e.g., ADOS scores). Two studies matched participants on only one feature (Kumazaki et al., 2015; Postorino et al., 2015), 14 studies matched participants on more than variable, in various combinations of the above listed variables (Amr et al., 2011; Andersson et al., 2013; Coffman et al., 2015; Harrop et al., 2015; Hartley & Sikora, 2009; Holtmann et al., 2007; Kopp & Gillberg, 2011; Lai et al., 2011; McLennan et al., 1993; Park et al., 2012; Rivet & Matson, 2011b [Study 1]; Rivet & Matson, 2011b [Study 2]; Rivet & Matson, 2011b [Study 3]; Solomon et al., 2012), and 14 studies did not specify if matching procedures were employed (Baghdadli et al., 2003; Bölte et al., 2011; Carter et al., 2007; Dworzynski et al., 2012; Frazier et al., 2014; Gadow et al., 2004a; Hattier et al., 2011; Head, 2014; Hess et al., 2010; Koyama et al., 2009; Kozlowski et al., 2012; Mandy et al., 2012; Reinhardt et al., 2015; Sipes et al., 2011). The most common variables used to match participants were age (41.9% included age as at least one variable) and IQ (25.8% included IQ as at least one variable). Only 4 of the 30 studies matched participants for ASD diagnosis (Holtmann et al., 2007; Kopp & Gillberg, 2011; McLennan et al., 1993; Rivet & Matson, 2011b [Study 3]).

Studies into sex-based variation in ASD have often used small female sample sizes in comparison to male samples. This aspect of the research is in part due to significantly fewer females being diagnosed with ASD. Insufficient female sample sizes limit the capacity for statistical comparisons to be made between the sexes due to a lack of power (Wing & Potter, 2002; Zwaigenbaum et al., 2007). Only 11 of the 30 studies considered in this review adopted equal sample sizes of males and females diagnosed with ASD (i.e., ratio of 1:1) (Andersson et al., 2013; Coffman et al., 2015; Harrop et al., 2015; Head, 2014; Holtmann et al., 2007; McLennan et al., 1993; Postorino et al., 2015; Rivet & Matson, 2011b [Study 1]; Rivet & Matson, 2011b [Study 2]; Rivet & Matson, 2011b [Study 3]; Solomon et al., 2012). Male-to-female ratios for the remainder of the studies ranged from 1.2:1 to 5.8:1 (Amr et al., 2011; Baghdadli et al., 2003; Bölte et al., 2011; Carter et al., 2007; Dworzynski et al., 2012; Frazier et al., 2014; Gadow et al., 2004a; Hartley & Sikora, 2009; Hattier et al., 2011; Hess et al., 2010; Kopp & Gillberg, 2011; Koyama et al., 2009; Kozlowski et al., 2012; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012; Park et al., 2012; Reinhardt et al., 2015; Sipes et al., 2011). Interestingly, of those studies that consisted of equal sample sizes, only 3 matched participants on age, IQ and ASD diagnosis (Holtmann et al., 2007; McLennan et al., 1993; Rivet & Matson, 2011b [Study 3]). This trend reflects the difficulties encountered by researchers who seek to match plus create equal sample sizes.

A number of researchers have argued that the sampling differences described above are (in part) exacerbated by the case ascertainment procedures used to recruit participants. Case ascertainment refers those methods of recruiting subjects for inclusion in a study. The applicability of findings to broader populations may be limited by the sources from which cases have been recruited, particularly when participants are obtained using existing administrative databases or national registers. Recruiting from specialist clinics and databases that are created for clinical purposes may result in higher numbers of participants

exhibiting lower severities (as in the case of treatment and intervention databases) or higher symptom severities (as would be expected in developmental screening databases) (Fombonne, 2009; Wing & Potter, 2002). Symptom severity may also be masked in those individuals who have also been exposed to prior treatment because intervention may reduce ASD-related behaviours and accelerate skill development (Zwaigenbaum et al., 2007). Participants who have been sourced from clinics and ASD-specific databases are more likely to have been exposed to treatment and intervention programs. In the studies included in this review, participants were recruited from a number of sources including: specialised clinics for developmental and other disorders, special education programs, ASD community organisations and support groups, previous research samples, intervention programs, population-based databases and ASD specific databases. Twenty studies sourced participants from clinically-based sources, such as clinics that specialised in Autism, Developmental and other medical/neuropsychiatric disorders, intervention programs, ASD specific community and support groups, and a national ASD Database (Amr et al., 2011; Andersson et al., 2013; Bölte et al., 2011; Gadow et al., 2004a; Hartley & Sikora, 2009; Hattier et al., 2011; Hess et al., 2010; Holtmann et al., 2007; Kopp & Gillberg, 2011; Koyama et al., 2009; Kozlowski et al., 2012; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012; McLennan et al., 1993; Postorino et al., 2015; Reinhardt et al., 2015; Rivet & Matson, 2011a [Study 2]; Rivet & Matson, 2011b [Study 3]; Solomon et al., 2012). For the remaining studies, participants were sourced from a previous research study sample (Harrop et al., 2015) population-based databases (1 genetics database, Frazier et al., 2014;) 1 twin database, (Dworzynski et al., 2012), and an ASD-based database (Park et al., 2012), 2 studies recruited from intervention programs (Rivet & Matson, 2011b [Study 1], Sipes et al., 2011), while 4 studies did not specify the recruitment sources (Baghdadli et al., 2003; Carter et al., 2007, Coffman et al., 2015; , Head, 2014).

There are a number of conclusions that can be drawn from the aforementioned methodological limitations (pages 131-137) relating to sampling methods. First, there are insufficient data to discuss a general male/female phenotype that applies across the lifespan. Second, conclusions regarding phenotype must be reported with caution and with references to particular developmental periods. Third, the research focus has been on early development and this reduces any comment on teen-adult phenotype variations.

The following discussion encompasses inter-study difficulties arising from variation in data-collection methods. Of particular importance to effective data collection is the process of triangulation: a method of increasing the validity of a study by incorporating two or more measurement methods to develop a comprehensive understanding of phenomena and for confirmation and generalisability of the research via minimisation of bias (Carter, Bryant-Lukosius, DiCenso, Blythe, & Neville, 2014; Heppner, Kivlighan, & Wampold, 1999; Mathison, 1988). The following discussion focused on data triangulation and environmental triangulation as these two factors related to the information collected to inform the exploration of sex-based ASD profiles. Data triangulation involves the collection of information from at least two sources, to reduce a study's vulnerability to the errors associated with a particular reporting method and to elicit rich information about the phenomena under investigation (Carter, Bryant-Lukosius, DiCenso, Blythe, & Neville, 2014; Patton, 1999). Data triangulation generally involves two components: (i) reporting sources and (ii) data sources.

Reporting sources refers to those individuals who provide data (i.e., reports of ASD symptoms and symptom severity), and may include any combination of self-reporters, independent raters (i.e., parents, teachers, care-staff), and clinical/professional raters (i.e., medical staff, psychiatrists, psychologists, paediatricians, and therapists). By using data from a number of informants to assess and examine behaviour, researchers can reduce systematic

reporting bias (De Los Reyes, et al., 2013). The majority of studies assessed in this review incorporated reports from multiple sources: 11 studies gathered information from 2 sources (Andersson et al., 2013; Bölte et al., 2011; Coffman et al., 2015; Gadow et al., 2004a; Head, 2014; Hess et al., 2010; Kopp & Gillberg, 2011; Kumazaki et al., 2015; McLennan et al., 1993; Park et al., 2012; Sipes et al., 2011), 10 studies from 3 sources (Amr et al., 2011; Baghdadli et al., 2003; Dworzynski et al., 2012; Frazier et al., 2014; Hartley & Sikora, 2009; Holtmann et al., 2007; Lai et al., 2011; Postorino et al., 2015; Solomon et al., 2012), 1 study from 4 sources (**157**), while 8 studies relied on a single data source (Harrop et al., 2015; Hattier et al., 2011; Koyama et al., 2009; Kozlowski et al., 2012; Reinhardt et al., 2015; Rivet & Matson, 2011b [Study 1]; Rivet & Matson, 2011b [Study 2]; Rivet & Matson, 2011b [Study 3]). The most commonly used data sources involved clinicians administering assessments to participants with this method being employed in 21 of the 30 studies (Amr et al., 2011; Andersson et al., 2013; Baghdadli et al., 2003; Bölte et al., 2011; Carter et al., 2007; Coffman et al., 2015; Frazier et al., 2014; Harrop et al., 2015; Hartley & Sikora, 2009; Holtmann et al., 2007; Kopp & Gillberg, 2011; Koyama et al., 2009; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012; McLennan et al., 1993; Park et al., 2012; Postorino et al., 2015; Reinhardt et al., 2015; Sipes et al., 2011; Solomon et al., 2012). Eighteen studies collected data via parent report (Amr et al., 2011; Carter et al., 2007; Dworzynski et al., 2012; Frazier et al., 2014; Gadow et al., 2004a; Hartley & Sikora, 2009; Head, 2014; Hess et al., 2010; Holtmann et al., 2007; Kopp & Gillberg, 2011; Kozlowski et al., 2012; Lai et al., 2011; Mandy et al., 2012; Park et al., 2012; Postorino et al., 2015; Rivet & Matson, 2011b [Study 1]; Rivet & Matson, 2011b [Study 2]; Solomon et al., 2012). Sixteen studies involved the administration of parent assessments by a clinician (Amr et al., 2011; Andersson et al., 2013; Baghdadli et al., 2003; Bölte et al., 2011; Carter et al., 2007; Coffman et al., 2015; Dworzynski et al., 2012; Frazier et al., 2014; Hartley & Sikora, 2009; Holtmann et al., 2007; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012;

McLennan et al., 1993; Postorino et al., 2015; Sipes et al., 2011). Interestingly, 19 of the 23 studies that employed clinician-driven data-collection failed to specify who the examiner was and if they had the appropriate qualification level to administer assessments that required a minimum level qualification, such as the Autism Diagnostic Interview (ADI) and Autism Diagnostic Observation Schedule (ADOS) (Bölte et al., 2011; Carter et al., 2007; Coffman et al., 2015; Dworzynski et al., 2012; Frazier et al., 2014; Harrop et al., 2015; Hartley & Sikora, 2009; Hess et al., 2010; Holtmann et al., 2007; Kopp & Gillberg, 2011; Koyama et al., 2009; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012; McLennan et al., 1993; Park et al., 2012; Postorino et al., 2015; Reinhardt et al., 2015; Solomon et al., 2012).

Data sources refer to the methods of gathering data, which may be done via interviews, observation, and document analysis (such as rating scales and questionnaires) (Carter et al., 2014; Heppner, Kivlighan, & Wampold, 1999). Data source inconsistencies concern the use of differing assessment measures to evaluate the presence and severity of symptoms across studies. Behavioural instruments developed to assist with diagnosis, such as the ADI and ADOS, seek to determine the presence of impairment and/or absence of skills as outlined in the diagnostic criteria for ASD (Payakachat, Tilford, Kovacs, & Kuhlthau, 2012). Standardisation samples for such instruments have consisted predominately of male subjects (Koenig & Tsatsanis, 2005; Rivet, & Matson, 2011a) and this raised concerns about the validity of such instruments in accurately identifying females with suspected ASD (and, prior to the DSM-5, the most appropriate possible diagnosis), thus making behavioural comparisons between the sexes difficult (Matson, Nebel-Schwalm, & Matson, 2007; Mandy et al., 2012; Rivet, & Matson, 2011a). In addition, and in part due to varying participant ages, differing assessments have been employed within the same study, which increases measurement error and fails to accurately capture behavioural differences between males and females (Carter et al., 2007). Outcomes may also depend on the manner in which assessment measures are

implemented. Retrospective reports are subject to errors of recall, particularly when a number of years have elapsed since the emergence of atypical behaviour. Caregiver reports and observations may be limited compared to systematic assessments conducted by clinicians. Further, where a diagnosis has been established, caregiver reports may be inadvertently exaggerated towards behaviours that are consistent with that diagnosis (Hus, Taylor, & Lord, 2011; Volkmar, Szatmari, & Sparrow, 1993).

In this review of 30 studies a total of 81 different assessment instruments/methods were used to measure variation in autism symptomology between males and females. As Table 2 (page 142) shows, the majority of studies incorporated the use of multiple assessments measures.

Table 2

Number of Assessment Measures Employed in Research Studies

Number of assessment measures employed	Number of Studies in which the assessment was used	Study References
1	6	Gadow et al., 2004a; Hattier et al., 2011; Head, 2014; Koyama et al., 2009; Rivet & Matson, 2011b [Study 1]; Sipes et al., 2011.
2	4	Amr et al., 2011; Kozlowski et al., 2012; Kumazaki et al., 2015; McLennan et al., 1993.
3	5	Dworzynski et al., 2012; Hess et al., 2010; Holtmann et al., 2007; Rivet & Matson, 2011b [Study 2]; Rivet & Matson, 2011b [Study 3].
4	3	Baghdadli et al., 2003; Harrop et al., 2015; Reinhardt et al., 2015.
5	5	Carter et al., 2007; Hartley & Sikora, 2009; Kopp & Gillberg, 2011; Mandy et al., 2012; Postorino et al., 2015.
6	1	Coffman et al., 2015.
7	1	Andersson et al., 2013.
8	2	Bölte et al., 2011; Solomon et al., 2012.
9	2	Lai et al., 2011, Park et al., 2012.
11	1	Frazier et al., 2014.

Those studies that incorporated multiple assessments did so using a variety of different data sources types. Examples of the types of assessments included interviews, ratings scales, task-based instruments, observation, questionnaires, neurological, cognitive, and personality testing, and checklists. Table 3 (page 143) below shows the assessment measures most commonly used to draw comparisons between male and female symptom profiles. These have been categorised into those that were used to compare autism symptoms (i.e., ADI, ADOS and diagnostic criteria), those that evaluated functional level (i.e., VABS), and those that assessed the presence of comorbid behaviour problems and associated difficulties (i.e., CBCL). The ADI, ADOS, CBCL and VABS, are all reported to have good psychometric properties (i.e., reliability and validity) and have been standardised. In addition, the ADI and

ADOS have been specifically designed to assess ASD symptomology and therefore correlate well with DSM-5 diagnostic criteria to ensure the accurate assessment of symptoms and symptom severity.

Table 3

Most Commonly Used Assessment Measures

Assessment Measure	Number of Studies in which the assessment was used	Type of Assessment	Study References
ADI ADI-R	7	Clinician Administered interview to Caregiver/Parent	Baghdadli et al., 2003; Bölte et al., 2011; Carter et al., 2007; Holtmann et al., 2007; Frazier et al., 2014; Lai et al., 2011; Park et al., 2012.
ADOS ADOS-2 ADOS-R ADOS-G	11	Clinician administered structure play session	Andersson et al., 2013; Bölte et al., 2011; Carter et al., 2007; Coffman et al., 2015; Frazier et al., 2014; Harrop et al., 2015; Hartley & Sikora, 2009; Holtmann et al., 2007; Lai et al., 2011; Mandy et al., 2012; Postorino et al., 2015; Reinhardt et al., 2015; Solomon et al., 2012.
DSM-IV-TR ICD-10 criteria	6	Checklist	Hartley & Sikora, 2009; Hess et al., 2010; Kopp & Gillberg, 2011; Park et al., 2012; Rivet & Matson, 2011b [Study 2]; Rivet & Matson, 2011b [Study 3].
VABS VABS-II VABS-SF	7	Parent-reports, teacher-report, clinician interview	Andersson et al., 2013; Baghdadli et al., 2003; Carter et al., 2007; Frazier et al., 2014; Hartley & Sikora, 2009; Postorino et al., 2015; Reinhardt et al., 2015.
CBCL	7	Parent-reports, teacher-report, self-report checklist	Amr et al., 2011; Bölte et al., 2011; Frazier et al., 2014; Hartley & Sikora, 2009; Holtmann et al., 2007; Park et al., 2012; Postorino et al., 2015.

While the use of such varied assessment measures across studies raises concerns over the capacity to draw comparisons regarding the male and female ASD profiles between studies, the use of various data source types and standardised measures suggests that

researchers are aware of the need to triangulate this aspect to accurately capture the subtle differences that may exist.

Finally, *environmental triangulation* involves the use of different locations/settings/contexts to collect data on the same participant features. Environmental factors, such as time of day, day of week, and location, have the potential to influence an informant's responding and affect data integrity (Guion, 2002). Of the 30 studies reviewed here, 27 failed to specify the contexts in which data were collected (i.e., clinical setting, participants home, research laboratory, Amr et al., 2011; Andersson et al., 2013; Baghdadli et al., 2003; Bölte et al., 2011; Carter et al., 2007; Coffman et al., 2015; Dworzynski et al., 2012; Frazier et al., 2014; Gadow et al., 2004a; Harrop et al., 2015; Hartley & Sikora, 2009; Hattier et al., 2011; Hess et al., 2010; Holtmann et al., 2007; Kopp & Gillberg, 2011; Koyama et al., 2009; Kumazaki et al., 2015; Lai et al., 2011; Mandy et al., 2012; McLennan et al., 1993; Park et al., 2012; Reinhardt et al., 2015; Rivet & Matson, 2011b [Study 1]; Rivet & Matson, 2011b [Study 2]; Sipes et al., 2011; Solomon et al., 2012). Of the remaining three studies, one reported the location to be a developmental centre (Rivet & Matson, 2011b [Study 3]), one indicated that data were collected predominantly in the home (Head, 2014), and the final study indicated that informants completed the measures either in their home or in a clinical setting (Kozlowski et al., 2012).

Despite the methodological inconsistencies discussed in this brief review of the research into sex-based phenotypic variation in ASD, a number of factors have been explored in relation to accurate identification of ASD in females. It has been proposed that females may demonstrate greater levels of autistic symptoms, present with additional behaviour problems, and/or experience greater cognitive impairment, compared to male counterparts (Andersson, Gillberg, & Miniscalco, 2013; Dworzynski et al., 2012).

6.2.2: Behavioural Differences between Males and Females with an ASD.

While the issue of females with an ASD presenting differently to their male counterparts has been acknowledged, no unified female profile has been proposed, largely due to inconclusive findings from previous research (Kirkovski, Enticott, & Fitzgerald, 2013; Lai et al., 2013; Saulnier & Ventola, 2012). That is, despite an increased interest in how symptom expression might be different in boys versus girls, it is important to acknowledge that studies in this area have not yet provided conclusive data regarding those possible differences (Kirkovski et al., 2013; Rivet, & Matson, 2011a; Van Wijngaarden-Cremers, Eeten, Groen, Deurzen, Oosterling, & Gaag, 2014). The most supported finding on male-female discrepancy in the ASD phenotype is the disproportionate sex ratio for overall diagnosis prevalence, which favours boys over girls at an average ratio of 4:1 (Dworzynski et al., 2012; Zwaigenbaum et al., 2012). Researchers have questioned this discrepancy in prevalence by suggesting that existing diagnostic criteria do not adequately represent the particular profile of symptoms and behaviours evident in females. That is, females with ASD may have a different phenotype to males with ASD. If found to be true, this insufficient understanding of ASD manifestation in females has important implications for the delivery of effective treatment to girls and women with ASD (Lai et al., 2013; Rivet, & Matson, 2011a).

Comparative studies which have sought to classify autism symptoms and behaviours on the basis of sex reveal some interesting findings. For example, males with ASD have been shown to engage in more obvious stereotyped behaviours (such as rocking or unusual object use) and to possess a greater number of unusual restricted interests (Hartley & Sikora, 2009) than females with ASD, who present with more subtle stereotyped behaviours (which tend to involve animals and people rather than objects) and restricted interests. In addition, these stereotyped behaviours and restricted interests appear to be more appropriate to the kinds of gender- and/or social expectations that are placed on females. Therefore, it is conceivable that

existing diagnostic criteria, which focus on the presence of overt behaviours and strong atypicality, might not detect more subtle female-oriented ASD symptoms.

Recent studies which have sought to identify sex-base variation in ASD have shown either (a) contrasting results or (b) no differences at all across the broad diagnostic constructs of socialisation/communication and RRBIs. These inconsistencies in findings across studies highlight the need to continue investigating ASD symptomatology in girls compared to boys, and to do so in a systematic manner, controlling for factors that are likely to mask the female ASD profile.

6.2.3: Biological Theories for Sex Differences in Autism.

Research into the manifestation of autism-related symptoms and associated behavioural features suggests that variation in these factors could (at least in part) be caused by sex differences. A growing body of evidence suggests that the female-specific profile of ASD symptomology may also be the result of underlying sexually dimorphic biological mechanisms. Biological theories attempting to account for the male–to-female disparity in ASD have implicated a number of genetic variants and environmental factors as possible contributors to liability (Van Wijngaarden-Cremers et al., 2014).

To date, no single genetic mechanism has been shown to account for the development of ASD, which indicates that genetic heterogeneity, epigenetic factors, multiple genes, and gene–gene and gene–environment interactions are all potential contributory factors (Szatmari et al., 2011). Genetic theories propose a multiple threshold multifactorial liability model, whereby numerous genes combine and interact to contribute to disease risk (Szatmari et al., 2011). The *additive model* suggests that females (because they are heterozygotic) have a higher threshold for becoming affected by ASD than males and therefore must inherit a higher genetic load than males before they reach that threshold (Lewis, 2002, Robinson, Lichtenstein, Anckarsäter, Happé, & Ronald, 2013; Werling & Geschwind, 2013). That is, it would take a greater number of autism risk genes for a female to exhibit ASD impairment. This higher genetic threshold also results in lower prevalence as fewer females are likely to be diagnosed with ASD compared to males, who are more likely to be diagnosed at lower thresholds (Szatmari et al., 2011). This difference in genetically-determined prevalence may also have led to the focus of ASD diagnostic criteria being placed on male symptoms rather than female symtoms. That is, lower genetic thresholds and resulting higher prevalence may have also contributed to a greater number of boys being included in the field trials used to develop diagnostic criteria, meaning that such criteria are predominately based on the male

presentation of ASD (Koenig & Tsatsanis, 2005). As identified by Dworzynski et al. (2012), females may go unrecognized when the clinical manifestations of ASD in girls compared to boys are not recognized.

Genome-wide association studies have implicated various genes located on the sex chromosomes and autosomes as potential contributors to the development of ASD (Auranen et al., 2002; Chung et al., 2011; Jamain et al., 2003; Liu et al., 2011). Genes located on the X-chromosome and those genes that are related to sex hormone functioning are believed to be strong candidates because they have been implicated in sexually dimorphic brain structure, neurochemistry, behaviour and susceptibility to neurodegenerative and neuropsychiatric disease (Stone et al., 2004; Trabzuni et al., 2013). X-linked genes known to show sex-based variation in expression and that escape X-inactivation may also be a factor in the sex-specific susceptibility to ASD (Schumann et al., 2010). Two loci on the X chromosomes that have been associated with a predisposition to autism include XP22.3 and Xq12-21 (Auranen et al., 2002; Jamain et al., 2003; Liu et al., 2011; Thomas et al., 1999). Thomas et al. (1999) reported *de novo* (spontaneously arising, Samocha et al., 2014) chromosomal deletions at Xp22.3 in three females who demonstrated features of ASD indicating that this may be a critical region for ASD in females. Thomas et al. (1999) also suggested that such presentation of autism-like symptoms may be due to a loss of gene function within the deleted region or a null allele (one that does not express a protein or the protein that is expressed lacks functionality, Hyde, 2009) resulting from X-inactivation of the normal X chromosome. Although data on the role of X-inactivation in ASD is limited (Kirkovski et al., 2013), the expression of symptoms associated with an abnormal X-linked gene may be suppressed when that gene is preferentially inactivated, and this may play a role in female-specific ASD symptomology (Matsuo et al., 1999; Robinson et al., 2013). In a study of 77 females (35 diagnosed with autism and 42 unaffected siblings), Talebizadeh et al. (2005) reported a higher degree of

skewed X-inactivation (which describes a bias toward cells expressing one or the other X chromosome when the inactivation of one is favoured over the other (Talebizadeh, Bittel, Veatch, Kibiryeva, & Butler, 2005) in affected subjects (33%) compared to controls (11%). Interestingly, of those affected females with highly skewed X-inactivation, 50% also had mothers with similar X-inactivation patterns, suggesting that skewed patterns may be genetically transmitted.

Neuroimaging studies designed to illuminate the role of brain structure in the development of ASD have also been used to explore the impact of sex-based variance in symptom manifestation. Given the obvious sex differences in typically developing male and female neuroanatomy, an exploration of similar possible differences in ASD may provide insight into the development of sex-specific symptom profiles between the sexes (Bloss & Courchesne, 2007; Calderoni et al., 2012). Emerging evidence does indeed suggest that sex differences also exist in the neuroanatomy and structural connectivity of the brains of males versus females with ASD (Beacher et al., 2012; Bloss & Courchesne, 2007; Calderoni et al., 2012; Schumann et al., 2010). Numerous neuroanatomical studies have investigated the extent of this variance between males and females with ASD as well as how females with ASD differ from typically-developing females. In a study of 58 children aged between 2 and 8 years (38 diagnosed with ASD, matched on age and non-verbal IQ with 19 control subjects), Calderoni et al (2012) used VBM to assess total intracranial volume (TIV), finding that females with ASD had approximately 5% greater TIV than controls and increased GM volume in the left superior frontal gyrus. Schumann et al (2010) conducted a longitudinal MRI study of 75 toddlers (ASD group: 32 males and 9 females; Control group: 32 males and 12 females), where data were collected at multiple time periods from 1.5 years up to 5 years of age, in order to assess brain growth trajectories from the time clinical ASD symptoms became apparent. Compared to controls, males with ASD showed significantly enlarged frontal and temporal

lobe GM volumes and cingulate GM grew at a non-linear rate. In females with ASD, greater abnormality in growth was observed in a greater number of brain regions, including total cerebrum, cerebral WM, cerebral GM, frontal, and temporal regions, while the cingulate GM was enlarged in females, but not males, with autism. In another study of 58 children (27 males and 9 females diagnosed with ASD and a control group consisting of 13 male and 14 female typically developing individuals) Bloss and Courchesne (2007) found males and females with ASD to exhibit similar abnormalities including whole brain enlargement, and in cerebral and frontal GM, and cerebellar WM volumes. It was also reported that girls with ASD showed additional abnormal enlargement in intracranial and temporal GM volumes and a significant reduction in cerebellar GM volume. Further, Beacher et al. (2012) assessed 58 adults (15 males and 13 females with ASD and a typically developing control group consisting of 15 males and 15 females) using structural MRI and calculated FA and mean diffusivity (MD). Sex differences were found for gross WM volume, regional GM volume, and FA in white matter which was greater for males than females in both groups, but these differences were reported to be less pronounced in the ASD group.

Overall, studies indicate that females with ASD differ from their male counterparts in relation to neuroanatomical profile, which may, be partly responsible for the observed differences in the suggested sex differences in the behavioural manifestation of ASD. However, further research is required to better understand the contribution of structural brain differences in sexually dimorphic symptomology (Chen, Van Horn, & GENDAAR Research Consortium, 2016). Kirkovski et al (2016) sought to explore the neural underpinnings of social understanding in ASD using fMRI. They investigated the Theory of Mind (ToM) hypothesis (which proposes that social/communication impairments of autism result from an individual's limited capacity to infer mental states to themselves and others, Rajendran & Mitchell, 2006). The fMRI data were collectedfrom brain regions that have been consistently

associated with ToM deficits; namely the medial prefrontal cortex (mPFC) and the tempro-parietal junction (TPj). The study sample consisted of 50 participants (13 males and 14 females with an ASD and a neurotypical group of 11 males and 12 females) aged between 19 and 56 years. While no group differences were identified in the regions of interest, males had decreased BOLD activity in the right posterior superior temporal sulcus (pSTS) compared to control males, with this trend not being observed in either of the female groups. As discussed by Kirkovski et al. (2016), impaired functioning of the STS has been consistently implicated in ASD research concerning ToM and sex differences. The underlying brain regions that mediate ToM may provide insight into the differential manner in which males and females present in regards to the socialisation domain. Understanding such differences could better inform treatment and intervention to achieve better outcomes for females with ASD who experience social difficulties.

Similarly to those differences noted in typical development, sexual dimorphism of the brain and subsequent behaviour between males and females with an ASD has been partly attributed to the differing hormone profiles between the sexes. Studies assessing such hormone underpinnings have implicated testosterone, estrogen, oxytocin, and vasopressin as possible contributors (Carter, 2007; Ruta, Ingudomnukul, Taylor, Chakrabarti, & Baron-Cohen, 2011). Sex-based differences in ASD traits may be brought about by the varied effects of hormones on gene expression, particularly those that are sensitive to gonadal hormones (Chakrabarti et al., 2009; Sarachana, Xu, Wu, & Hu, 2011), by influencing brain function directly (by coding for proteins that interact with receptors in the brain) or indirectly (by coding for proteins that affect other bodily tissues) (Nguyen, Rauch, Pfeifer, & Hu, 2010; Schumann et al., 2010). Sarachana, Xu, Wu and Wu (2011) identified the retinoic acid-related orphan receptor-alpha (RORA) gene as a potential candidate for sex bias in ASD, given its involvement in a number of functions that are impacted in this condition, including regulation

of circadian rhythms, neuroprotection during oxidative stress and inflammation, survival and differentiation of Purkinje cells, and cerebellar development (Nguyen et al., 2010). RORA is a hormone-dependent transcription factor that regulates the transcription of aromatase (Nguyen et al., 2010). The expression of RORA is differentially modulated by male- and female-specific sex hormones through differential regulatory pathways that create a feedback loop. Sarachana et al. (2011) found that binding to the RORA promoter regions in the brain is increased through the regulatory relationship of DHT with androgen receptors and oestradiol with oestrogen receptors. Specifically, oestradiol enhances RORA expression and DHT represses the expression of RORA, suggesting this as a potential mechanism for the sex bias in autism. However, the contribution of such epigenetic effects on male- and female-specific behavioural traits in autism was not explored in that study and thus requires further investigation.

Hormone theories, such as the androgen theory of ASD, propose that exposure to increased levels of prenatal and neonatal androgens may contribute to the sexually dimorphic expression of ASD traits in a similar manner to sex differentiated behaviour observed in neurotypical individuals through differential brain development (Knickmeyer & Baron-Cohen, 2006; Ruta et al., 2011; Schwarz et al., 2011). In a study that aimed to explore the relationship between foetal testosterone (fT, measure by extracting amniotic fluid) levels and ASD traits in a group of neurotypical children, Auyeung et al. (2009) assessed 235 subjects (118 boys and 117 girl) aged between 6 and 10 years. That research team found increased levels of fT in boys was found to be positively associated with higher scores on the Childhood Autism Spectrum Test (CAST) and the Child Autism Spectrum Quotient (AQ-Child), suggesting that higher levels of fT may have a causal effect on neural development and ASD traits. Schwartz et al. (2011) reported similar results in a study of 166 adults (33 males and 29 females diagnosed with ASD and a control sample consisting of 49 males and 55 females).

Testosterone was measured through blood serum and ASD traits were assessed with the AQ-Adult. Typically developing males exhibited higher levels of testosterone than their neurotypical female counterparts, while similar levels of testosterone were noted between males and females in the ASD group. Such results may be interpreted as indicating that males and females with ASD have distinct biomarker fingerprints, implicating androgen as a factor in the development of ASD in both males and females.

While there is agreement as to the potential for underlying biological determinants in autism, the contributions of specific genetic, hormone and environment influences on sex differences in ASD behavioural profiles remain largely unknown and require further systematic investigation if they are to inform the assessment, diagnosis and subsequent treatment of ASD between the sexes (Schumann et al., 2010).

6.3: Researching Sex Differences in Autism: Conclusion

This thesis has presented evidence on the longevity and pervasiveness of sex differences across species and within humans with particular reference to sex-based variation in morphology, physiology and behaviour (McPherson & Chenoweth, 2012**;** Wizemann, & Pardue, 2001). Interestingly, whilst biologically-oriented sex differences are well-established and confirmed by many decades of research, the male-to-female differences which pertain to social behaviour and (in particular) mental disorder are not as uniformly supported. Researchers have suggested that any conclusion regarding male *versus* female behaviour and responses to mental disorder are complicated by the impacts of gender and the difficulties in adequately differentiating between this socially-influenced aspect of functioning and basic genetic and biological influences. Despite this complexity, there is general agreement among researchers that the differing representation of males to females in the onset of mental disorders such as anxiety and depression, as well as disparities in symptom expression in daily life, provide evidence of underlying biological differences between men and women (Afifi, 2007; Pankevich et al., 2011). The research on mental disorder which suggests that sex and gender interact to produce differences in risk and susceptibility, onset and course, treatment-seeking behaviour, treatment responsiveness, and subsequent psychological adjustment, also argues for investigation into and development of sex/gender differentiated assessment and therapy methods to address the particular experiences and therapeutic needs of males *versus* females (Bao & Swaab, 2010; WHO, 2015; Wizemann, & Pardue, 2001).

The issue of how sex differences might influence symptom expression and impairment patterns of children with neurodevelopmental disorders such as ASD has become increasingly significant in the clinical and educational fields. It is clear that neurodevelopmental disorders of childhood result from the combined influence of genetic predisposition, brain abnormalities and environmental factors which in turn create the communication-social,

behavioural, and cognitive impairment pattern characteristic of ASD (Getz, 2014; Zahn-Waxler, Shirtcliff, & Marceau, 2008). Despite some variation in the current estimates of ASD prevalence (i.e., 1 child in 68 for the USA, Baio, 2014; 1 child in 100 in Australia, Buckley, 2013), the gender ratio of this disorder has remained at approximately 4 boys to 1 girl (Dworzynski et al., 2012; Zwaigenbaum et al., 2012). However, clinical research focused on methods for reliable detection of ASD indicates that the current incidence estimates under-represent cases of ASD in girls because their impairment profile differs to that of boys (Lai et al., 2013; Rivet, & Matson, 2011a; Van Wijngaarden-Cremers et al., 2014). Inspection of the research into ASD diagnostic procedures reveals that, whilst there is growing recognition that a male- versus female-specific ASD profile might exist, there are no current findings which describe robust guidelines for sex-based differentiation in symptom expression, disruptions to functioning, course, and prognosis (Rivet, & Matson, 2011a). Therefore, and to aid early detection and timely access to specialised interventions, further investigation which clarifies these issues with specific reference to the experiences of girls would be of benefit to the research, clinical, and educational fields. Incorporation of any relevant biological and socio-cultural factors in the assessment process could advance creation of behaviour profiles for females and males on the autism spectrum (Pilowsky, Yirmiya, Shulman, & Dover, 1998).

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Appendicies

Appendix A

Table A1

30 studies representing investigations into the female-male variation in ASD

STUDY COMPARISONS								
Article Reference	***Sampling and Sampling Methods***				***Assessment Measures***		***Data Collection***	
	Participant Characteristics	***Sample Characteristics***	***Matching Strategies***	***Case Ascertainment***	***Confirmation of Diagnosis***	***Use in Study***	***Data Sources***	***Reporting Sources***
STUDIES ALLOCATED TO "TODDLER" ANALYSIS GROUP								
Hartley & Sikora, 2009	*Aged:* 1.5-3.9 yrs. *Diagnosis*: DSM-IV-TR AD: 133 PDD-NOS: 66	199 (m=157 / f=42) Ratio: 3.7:1 78.89% : 21.11%	*Matched*: Age & IQ. *Exclusion criteria*: Unknown.	*Sourced from*: Interdisciplinary Autism clinic in a tertiary hospital.	*Confirmed With*: ADOS-G DSM-IV-TR Interview	*ADOS-G*: Autism symptoms. *CBCL*: Coexisting behaviour problems. *DSM-IV-TR Criteria*: Autism symptoms. *MSEL*: Developmental functioning (Cognitive & Motor). *VABS-II*: Adaptive behaviour (Personal and Everyday Living Skills).	Points of Data Collection Single. Data Sources *ADOS-G*: INT/TB/OBS/RS *CBCL*: CHK *DSM-IV-TR*: CHK *MSEL*: INT/TB/OBS *VABS-II*: INT Data Context (Environment) Not Specified.	*Parent Report*: CBCL *Clinician Assessment (Participant)*: ADOS-G: Examiner Not Specified. DSM-IV-TR: Examiner Not Specified. MSEL: Examiner Not Specified. *Clinician Administered (Parent)*: VABS-II: Examiner Not Specified. DSM-IV-TR: Examiner Not Specified.
Carter et al., 2007	*Aged:* 1.5-2.7 yrs. *Diagnosis*: DSM-IV (ADI-R) An ASD	90 (m=68 / f=22). Ratio: 3.1:1 m=75.55% f= 24.44%	*Matched*: Unknown. *Exclusion criteria*: Comorbid genetic, medical or neurological disorders.	*Sourced from*: Information not provided.	*Confirmed With*: ADI-R ADOS	*ADI-R:* Confirm diagnosis, Autism symptoms. *ADOS*: Confirm diagnosis, Autism symptoms. *ITSEA*: Social functioning. *MSEL*: Cognitive & developmental functioning. *VABS*: Social functioning.	Points of Data Collection Single. Data Sources *ADI-R*: INT *ADOS*: INT/TB/OBS/RS *ITSEA*: RS *MSEL*: INT/TB/OBS *VABS*: INT Data Context (Environment) Not Specified.	*Parent Report*: ITSEA *Clinician Assessment (Participant)*: ADOS: Examiner Not Specified. MSEL: Examiner Not Specified. *Clinician Administered (Parent)*: ADI-R: Examiner Not Specified. VABS: Examiner Not Specified.

Anderson et al., 2013	*Aged:* <4 years. *Diagnosis*: DSM-IV AD: M11; F9 = 20 ASD: M6; F9 = 15 AD: M1; F1 - 2 Autistic Traits: M2; F1 = 3	40 (m=20 / f=20). Ratio: 1:1 m= 50.00% f= 50.00%	*Matched*: Age & DQ. *Exclusion criteria*: Unknown.	*Sourced from*: Child Neuropsychiatry Clinic (referred for assessment).	*Confirmed With*: Griffiths' Developmental Scales RDLS WPPSI-III VABS ADOS ADOS-R CDIs CGAS DISCO-11	*ADOS:* Confirm diagnosis, Autism symptoms. *ADOS-R:* Confirm diagnosis, Autism symptoms (RRB). *CGAS*: General social & Psychiatric functioning. *GMDS*: Rate of development. *RDLS-III*: Language comprehension. *VABS*: Adaptive Behaviour (Personal and Everyday Living Skills). *WPPSI-III:* Verbal & performance intelligence.	Points of Data Collection Single. Data Sources *ADOS/ADOS-R*: INT/TB/OBS/RS *CGAS*: RS *GMDS*: INT/TB/OBS RDLS-III: INT/TB/OB *VABS*: INT *WPPSI-III*: INT/TB/OBS Data Context (Environment) Not Specified.	*Clinician Assessment (Participant)*: ADOS/ADOS-R GMDS WPPSI-III All measures administered by Researchers (Qualifications unknown). *Clinician Administered (Parent)*: VABS RDLS-III CGAS All measures administered by Researchers (Qualifications unknown).
Reinhardt et al, 2015	*Aged:* Mean 1.7 yrs. *Diagnosis*: DSM-IV (ADOS) An ASD	*ASD*: 288 (m=234 / f=54). Ratio: 4.3:1 m= 81.25% f= 18.75% *TD*: 223 (m=164 / f=59). Ratio: 2.8:1 m= 73.54% f= 26.46%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Tertiary Autism Clinic and Autism and Communication Disorders Clinic	*Confirmed With*: ADOS MSEL VABS	*ADOS*: Confirm diagnosis, Autism symptoms. *CBCS*: Early social communication abilities. *MSEL*: Confirm diagnosis, Developmental functioning. *VABS*: Confirm diagnosis, Adaptive Behaviour.	Points of Data Collection Single. Data Sources *ADOS*: INT/TB/OBS/RS *CBCS*: TB/CHK *MSEL*: INT/TB/OBS *VABS*: INT Data Context (Environment) Not Specified.	*Clinician Assessment (Participant)*: ADOS: Examiner Not Specified. CBCS: Examiner Not Specified. MSEL: Examiner Not Specified. VABS: Examiner Not Specified.
Postorino et al, 2015	*Aged:* 2-5.4 yrs (Mean age: 3.5 yrs). *Diagnosis*: DSM-IV-TR An ASD	*ASD*: 60 (m=30 / f=30). Ratio: 1:1 m=50.00% f=50.00%	*Matched*: Age. *Exclusion criteria*: Comorbid genetic or medical disorders and epilepsy.	*Sourced from*: Child Neuropsychiatry Clinic (referred).	*Confirmed With*: DSM-IV-TR criteria (assessed by expert).	*ADOS-G*: Autism Symptoms. *CBCL*: Emotional and behaviour problems *GMDS-ER*: Developmental quotient. *PSI-SF*: Parental stress. *VABS-SF*: Adaptive skills	*Points of Data Collection*: Multiple (2). Time range not specified. Data Sources *ADOS-G*: INT/TB/OBS/RS *CBCL*: CHK *GMDS-ER*: INT/TB/OBS *PSI-SF*: RS *VABS-SF*: INT Data Context (Environment) Not Specified.	*Parent Report:* CBCL PSI-SF *Clinician Assessment (Participant):* ADOS-G: Licenced clinician. *Clinician Administered (Parent)*: VABS-SF: Trained & experienced clinician. GMDS-ER Examiner Not Specified.

Harrop et al, 2015	*Aged:* 1.8 – 4.5 yrs (Mean age: 3.3 yrs). *Diagnosis*: DSM-IV (ADOS) An ASD	80 (m=40 / f=40). Ratio: 1:1 m= 50.00% f=50.00%	*Matched*: Study allocation and ADOS module/algorithm scores; *Exclusion criteria*: Unknown.	*Sourced from*: Previous tertiary based ASD research studies.	*Confirmed With*: ADOS-2	*ADOS-2*: Confirm diagnosis, Matching participants *ESCS*: Early verbal and non-verbal communication skills. *MSEL*: Ascertain language and non-verbal development. *SPA*: Play ability.	Points of Data Collection Single. Data Sources *ADOS-2*: INT/TB/OBS/RS *ESCS*: INT/TB/OBS *MSEL*: INT/TB/OBS *SPA*: INT/TB/OBS Data Context (Environment) Not Specified.	*Clinician Assessment (Participant):* ADOS-2: Examiner Not Specified. ESCS: Examiner Not Specified. MSEL: Examiner Not Specified. SPA: Examiner Not Specified.
Rivet & Matson, 2011b (Study 1)	*Aged:* 1.4-3.0 yrs. *Diagnosis*: DSM-IV-TR Autism PDD-NOS	*ASD*: 132 (m=66 / f=66). Ratio: 1:1 m= 50.00% f= 50.00% *TD*: 132 (m=66 / f=66). Ratio: 1:1 m= 50.00% f= 50.00%	*Matched*: Developmental variables (e.g., DQ, age, epilepsy, & ethnicity). *Exclusion criteria*: Individual prescribed psychotropic medications.	*Sourced from*: Early intervention program for children with medical conditions likely to result in developmental delay.	*Confirmed With*: DSM-IV-TR criteria M-CHAT BDI-2	*BISCUIT*: Autism symptoms.	Points of Data Collection Single. Data Sources *BISCUIT*: RS Data Context (Environment) Not Specified.	*Clinician Administered (Parent):* BISCUIT: Examiner Not Specified.
Sipes et al, 2011	*Aged:* 1.4-3 yrs. *Diagnosis*: DSM-IV-TR AD PDD-NOS	390 (m=294 / f=96). Ratio: 3.1:1 m= 75.38% f= 24.62%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Early intervention program.	*Confirmed With*: DSM-IV-TR criteria M-CHAT BDI-2	*BDI-2*: Developmental Skills. *BISCUIT*: Autism Symptoms.	Points of Data Collection Single. Data Sources *BDI-2*: PT; RS *BISCUIT*: RS Data Context (Environment) Private, quiet room away from distractions. Not Specified.	*Clinician Assessment (Participant):* BDI-2: Trained professionals. *Clinician Administered (Parent)*: BISCUIT: Trained professionals.
STUDIES ALLOCATED TO "CHILDREN" ANALYSIS GROUP								
Dworzynski et al, 2012	*Aged:* 10-12 yrs. *Diagnosis*: DSM-IV/ICD-10 (DAWBA) ASD	*ASD*: 189 (m=160 / f=29). Ratio: 5.5:1 m= 84.66% f= 15.34% *High CAST Group*: 174 (m=119 / f=55). Ratio: 2.2:1 m= 68.39% f= 31.61%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: population study of twins born in 1994 to 1996.	*Confirmed With*: DAWBA	*CAST*: Screen for ASD traits. DAWBA: Autism symptoms. *SDQ*: Behaviour problems.	Points of Data Collection Single. Data Sources *CAST*: QU/CHK *DAWBA*: INT *SDQ*: QU Data Context (Environment) Not Specified.	*Parent Report:* CAST *Teacher Report*: SDQ *Clinician Administered (Parent):* DAWBA: Examiner Not Specified.

Mandy et al, 2012	*Aged:* Mean (m=9.7 / f = 10.2). *Diagnosis*: DSM-IV-TR ASD: M81; F13 = 94 AD: M97; F16 = 113 PDD-NOS: M95; F23 = 118	325 (m=273 / f=52). Ratio: 5.3:1 m= 84.00% f= 16.00%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Specialist clinic for HF children with social-communication difficulties (referrals).	*Confirmed With*: 3Di ADOS (where available) Structured reports from school/nursery	*3Di*: Confirm diagnosis, Autism symptoms. *ADOS*: Autism symptoms. *British Picture Vocabulary Scale/ WASI-II/WISC-III/WISC-IV*: IQ. *SDQ*: Problem behaviour.	Points of Data Collection Single. Data Sources *3Di*: INT *ADOS*: INT/TB/OBS/RS *British Scale*: INT *SDQ*: QU *WASI-II/WISC-III/WISC-IV*: INT/TB/OBS Data Context (Environment) Not Specified.	*Parent and Teacher Report:* SDQ (Not available for all participants) *Clinician Administered (Participant):* ADOS: Psychologists at masters level and above, supervised by research reliable clinical psychologists. British Scale/WASI-II/WISC-III/WISC-IV: Examiner Not Specified. *Clinician Administered (Parent):* 3Di: Experienced child psychiatrist or clinical psychologist.
Koyama et al, 2009	*Aged*: Mean (m=9.0 / f=8.2 yrs). *Diagnosis*: DSM-IV AD: M21; F6 = 27 AS: M27; F4 = 31 PDD-NOS: M68; F16 = 84	142 (m=116 / f=26). Ratio: 4.5:1 m= 81.69% f= 18.31%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Specialist clinics for developmental disorders	*Confirmed With*: CARS-TV (symptom severity)	*WISC-III (Japanese Version)*: IQ.	Points of Data Collection Single. Data Sources *WISC-III (JP)*: INT/TB/OBS Data Context (Environment) Not Specified.	*Clinician Administered (Participant):* WISC-III (Japanese Version): Examiner Not Specified.
Amr et al, 2011	*Aged:* 4-11 yrs. *Diagnosis*: DSM-IV-TR AD: M33; F22 = 55 PDD-NOS: M4; F1 = 5	60 (m=37 / f=23). Ratio: 1.6:1 m= 61.66% f= 38.34%	*Matched*: Age & IQ. *Exclusion criteria*: Unknown.	*Sourced from*: Clinic-Centre for Early Diagnosis of Children's Disabilities	*Confirmed With*: DSM-IV-TR criteria interview ISAA	*CBCL*: Behaviour problems. *ISAA*: Autism symptom severity.	Points of Data Collection Single. Data Sources *CBCL*: CHK *ISAA*: INT/OBS Data Context (Environment) Not Specified.	*Parent Report:* CBCL *Clinician Administered (Participant) & Clinician Administered (Parent):* ISAA: Independent qualified child psychologist.
Kumazaki et al, 2015	*Aged:* 5-9 yrs. *Diagnosis*: DSM-IV AD: M4; F3 = 7 AS: M22; F17 = 39	46 (m=26 / f=20). Ratio: 1.3:1 m= 56.52% f= 43.48%	*Matched*: FIQ: 70. *Exclusion criteria*: FIQ below 70, Neurological & psychiatric problems.	*Sourced from*: Specialist Clinic for Developmental Disorders.	*Confirmed With*: Diagnostic Instruments and Screening questionnaires including the PARS	*CARS-TV (Tokyo version)*: degree & profiles of Autism. *WISC-III (Japanese Version):* IQ.	Points of Data Collection Single. Data Sources *CARS-TV*: RS *WISC-III (JP)*: INT/TB/OBS Data Context (Environment) Not Specified.	*Clinician Administered (Participant):* WISC-III (Japanese Version): Examiner Not Specified. *Clinician Administered (Informant):* CARS-TV (Tokyo version): Informant Not Specified.

Solomon et al, 2012	*Aged:* 8-11 yrs. *Diagnosis*: DSM-IV-TR HFA AS PDD-NOS	*ASD*: 40 (m=20 / f=20). Ratio: 1:1 m= 50.00% f= 50.00% *TD*: 36 (m=17 / f=19). Ratio: 1.1:1* m= 47.22% f= 52.78%	*Matched*: Age & IQ. *Exclusion criteria*: depression, anxiety disorders, ADHD, Fragile X, Tourette's, or seizure disorders. Diagnosed learning disabilities were not exclusionary.	*Sourced from*: local physicians, psychologists, speech-language pathologists, occupational therapists, advocacy and ADS support groups, regional centres (developmentally disabled), and Tracking System	*Confirmed With*: WASI ADOS SCQ	*ADOS-G*: Confirm diagnosis, Autism symptoms. BASC2: Adaptive & problem behaviours. *CCC-2*: Communication. *CDI*: Severity of depression. *RBS-R*: RRBI. *SCQ*: Qualification measure, Communication & social skills. *SRS*: Autism symptom Severity. *WASI*: Qualification measure, IQ.	Points of Data Collection Single. Data Sources *ADOS-G*: INT/TB/OBS/RS *BASC2*: BRS *CCC-2*: CHK *CDI* :PT/RS *RBS-R*: RS *SCQ*: RS *SRS*: BRS *WASI*: INT/TB/OBS Data Context (Environment) Not Specified.	*Self-Report (Participants):* CDI *Parent Report:* SCQ SRS BASC-2 CCC-2: Informant Not Specified. RBS-R: Informant Not Specified. *Clinician Administered (Participant):* WASI: Examiner Not Specified. ADOS: Examiner Not Specified.
Baghdadli et al, 2003	*Aged*: 2-7 yrs. *Diagnosis*: ICD-10 An ASD	222 (m=183 / f=39). Ratio: 4.7:1 m= 82.43% f= 17.57%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Unknown.	*Confirmed With*: ICD-10 criteria ADI-R CARS VABS	*ADI-R*: Expressive speech. *CARS*: Autism symptom severity. *SIB-Questionnaire*: Self-injurious behaviour. *VABS*: Adaptive behaviour & ID.	Points of Data Collection Single. Data Sources *ADI-R*: INT *CARS*: RS *SIB*: QU *VABS*: INT Data Context (Environment) Not Specified.	*Care-Staff Report:* SIB *Clinician Administered (Participant):* *ADI-R*: Psychologist/Psychiatrist who knew the participant best. *CARS*: Trained rater, based on video of child interacting with an adult. *Clinician Administered (Parent):* VABS: Trained professional.
Coffman et al, 2015	*Aged:* 8.3-14 yrs. *Diagnosis*: DSM-IV-TR ASD	24 (m=12 / f=12). Ratio: 1:1 m= 50.00% f= 50.00%	*Matched*: Age & DQ. *Exclusion criteria*: seizures, prior serious head injury, sensory or motor impairment, neurological disease, active psychiatric disorder, medication that affects brain electrophysiology, FSIQ <60.	*Sourced from*: Unknown.	*Confirmed With*: ADI-R ADOS DSM-IV-TR criteria	*ADOS*: Autism symptom severity. *BFRT*: Face perception (via EEG-Electrophysiological markers of social perception) *CSI-4*: Exclusionary screen. *DAS-II*: IQ. *VABS*: Adaptive behaviour. *WASI*: IQ.	Points of Data Collection Single. Data Sources *ADOS*: INT/TB/OBS/RS *BFRT*: NA/CA *CSI-4*: RS *DAS-II*: INT/TB/OBS *VABS*: INT *WASI*: INT/TB/OBS Data Context (Environment) Not Specified.	*Clinician Administered (Participant):* ADOS: Examiner Not Specified. BFRT: Examiner Not Specified. EEG: Examiner Not Specified. *Clinician Administered (Parent):* VABS: Examiner Not Specified.

Gadow et al, 2005	*Aged:* Mean 8.0 – 8.9 yrs. *Diagnosis*: DSM-IV AD: 67 AS: 24 PDD-NOS: 91	*Parent Report* *PDD*: 284 (m=242 / f=42). Ratio: 5.8:1 m= 85.21% f= 14.79% *NonPDD*: 189 (m=135 / f=54). Ratio: 2.5:1 m= 71.43% f= 28.57% *Spec.Ed*: 61 (m=38 / f=23). Ratio: 1.7:1 m= 62.30% f= 37.30% *Reg.EC*: 385 (m=186 / f=199). Ratio: 1.1:1* m= 48.31% f= 51.69% *Teacher Report* *PDD*: 284 (m=241 / f=43). Ratio: 5.6:1 m= 84.86% f= 15.14% *NonPDD*: 181 (m=127 / f=54). Ratio: 2.4:1 m= 70.17% f= 29.83% *Spec.Ed*: 60 (m=38 / f=22). Ratio: 1.7:1 m= 63.33% f= 36.67% *Reg.EC*: 404 (m=207 / f=197). Ratio: 1.1:1 m= 51.24% f= 48.76%	*Matched*: Unknown. *Exclusion criteria*: a diagnosis of communication disorder or undifferentiated communication disorder/ PDD were excluded from the PDD sample, whereas only the latter were excluded from the non-PDD clinic sample	*Sourced from*: Clinic Samples sourced from Tertiary hospital child psychiatry outpatient clinic. Community Samples sources from regular or special early childhood programs.	*Confirmed With*: interviews with the children/caregivers; informal observation of parent–child interaction; school reports, psycho-educational & special education evaluations; questionnaire of developmental, educational, medical, & family histories; & scores from several parent- and teacher-completed behaviour ratings scales including the ECI-4	*ECI-4:* Emotional & behavioural disorders.	Points of Data Collection Single. Data Sources *ECI-4*: RS Data Context (Environment) Not Specified (Parents sent questionnaires and distribute forms to teachers).	*Parent Report & Teacher Report:* ECI-4

STUDIES ALLOCATED TO "ADOLESCENTS" ANALYSIS GROUP								
Bölte et al, 2011	*Aged*: Mean *ASD* (m=14.0 / f=14.3). *SIB* (m=14.4 / f=14.8). *Diagnosis*: ICD-10 Autism: M24; F14 = 38 AS: M6; F5 = 11 PDD-NOS: M5; F2 = 7	*ASD*: 56 (m=35 / f=21). Ratio: 1.7:1 m= 62.50% f= 37.50% *SIB*: 58 (m=23 / f=35). Ratio: 1.5:1* m= 39.66% f= 60.34%	*Matched*: Unknown. *Exclusion criteria*: IQ < 70, lack of testability, lack of functional language and severe co-morbid medical conditions	*Sourced from*: Child and adolescent psychiatry departments (referred to as inpatients or outpatients) or a request in the journal of Autismus Deutschland.	*Confirmed With*: ADi-R ADOS	*ADI-R*: Confirm diagnosis, Autism symptoms. *ADOS*: Confirm diagnosis, Autism symptoms. *CBCL*: General psychopathology. *EFT*: Visual attention to detail (EF). *ToH*: Not specified (EF) *TMT-B-A*: speed of attention, conceptual tracking capacity (EF) *WCST*: Forming abstract concepts, shift sets, & usage of feedback (EF). *YABCL*: General psychopathology.	Points of Data Collection Single. Data Sources *ADI-R*: INT *ADOS*: INT/TB/OBS/RS *CBCL*: CHK *EFT*: NA/CA/TB *ToH*: NA/CA/TB *TMT*-B-A: NA/CA/TB *WCST*: NA/CA/TB *YABCL*: CHK Data Context (Environment) Not Specified	*Clinician Administered (Participant):* ADOS: Examiner Not Specified. EFT: 3 experienced clinical psychologists (randomised). ToH: 3 experienced clinical psychologists (randomised). TMT-B-A: 3 experienced clinical psychologists (randomised). WCST: 3 experienced clinical psychologists (randomised). *Clinician Administered (Parent):* ADI-R: Trained child psychiatrists. CBCL: Trained child psychiatrists. YABCL: Trained child psychiatrists.
Head et al, 2014	*Aged:* 10-16 yrs. *Diagnosis*: Criteria Not Specified ASD	*ASD*: 50 (m=25 / f=25). Ratio: 1:1 m= 50.00% f= 50.00% *TD*: 51 (m=26 / f=25). Ratio: 1.04:1 m= 50.98% f= 49.02%	*Matched*: Unknown. *Exclusion criteria*: LF Autism, PDD-NOS, ID.	*Sourced from*: Variety of sources including ASD support groups and databases.	*Confirmed With*: Unknown.	*FQ*: Friendship quality, understanding & empathy.	Points of Data Collection Single. Data Sources *FQ*: QU Data Context (Environment) Location and Time of participants choosing, predominantly in private homes.	*Self-Report*: FQ *Parent Report:* FQ

STUDIES ALLOCATED TO "ADULTS" ANALYSIS GROUP								
Lai et al, 2011	*Aged:* 18-45 yrs. *Diagnosis*: DSM-IV/ICD-10 AD AS	83 (m=45 / f=38). Ratio: 1.2:1 m= 54.22% f= 45.78%	*Matched*: Age & IQ. *Exclusion criteria*: Comorbid psychiatric and medical conditions.	*Sourced from*: national & local autism support organization, referral from diagnostic clinics for adults with autism, and via the participant database of the Autism Research Centre	*Confirmed With*: ADI-R ADOS	*ADI-R:* Eligibility, Autism symptoms *ADOS (Module 4)*: Eligibility, Autism symptoms. *AQ*: Autistic traits in social skills, attention switching, ATTD, communication, & imagination. *BAI:* Comorbid psychopathology. *BDI:* Comorbid psychopathology. *EQ*: Empathy. *EYES TEST*: Advanced mentalising (EF). *OCI-R*: Comorbid psychopathology. *SQ*: Systemizing. *WASI-II*: IQ.	Points of Data Collection Single. Data Sources *ADI-R*: INT *ADOS*: INT/TB/OBS/RS *AQ*: QU *BAI*: PT/RS *BDI*: PT/RS *EQ*: QU *OCI*-R: RS *SQ*: QU *WASI-II*: INT/TB/OBS Data Context (Environment) Not Specified.	*Self-Report:* AQ BAI BDI EQ OCI-R SQ *Clinician Administered (Participant):* ADOS: Examiner Not Specified. EYES TEST: Examiner Not Specified. WASI-II: Examiner Not Specified. *Clinician Administered (Parent):* ADI-R: Examiner Not Specified.
Rivet & Matson, 2011b (Study 3)	*Aged*: 18-87 yrs. *Diagnosis*: DSM-IV-TR AD PDD-NOS	*ASD*: 116 (m=58 / f=58). Ratio: 1:1 m= 50.00% f= 50.00% *TD*: 116 (m=58 / f=58). Ratio: 1:1 m= 50.00% f= 50.00%	*Matched*: Gender and ASD diagnosis. *Exclusion criteria*: Comorbid genetic, psychiatric disorders and unspecified severity of ID.	*Sourced from*: Developmental Centres	*Confirmed With*: DSM-IV-TR checklist.	*ASD-DA*: Autism symptoms. *DSM-IV-TR:* Autism symptoms. *ICD-10 Checklist*: Autism symptoms.	Points of Data Collection Single. Data Sources *ASD-DA*: RS *DSM-IV-TR*: CHK *ICD-10*: CHK Data Context (Environment) Developmental Centre.	*Residential Support Staff Report:* ASD-DS: Clinical psychology doctoral students. DSM-IV-TR: Clinical psychology doctoral students.
Hattier et al, 2011	*Aged*: Mean 49 yrs. *Diagnosis*: DSM-IV-TR An ASD (with severe to profound ID).	140 (m=77 / f=63). Ratio: 1.2:1 m= 55.00% f= 45.00%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Developmental Centre	*Confirmed With*: Unknown.	*DASH-II*: screen for symptoms of psychopathology in adults with severe to profound ID.	Points of Data Collection Single. Data Sources *DASH-II*: RS Data Context (Environment) Not specified.	*Care-Staff Report:* DASH-II: Examiner held a master's degree in a health related discipline.

STUDIES ALLOCATED TO "ACROSS THE LIFESPAN" ANALYSIS GROUP								
Frazier et al, 2014	*Aged*: 4-18 yrs. *Diagnosis*: DSM-IV-TR Autism ASD AS PDD-NOS	2418 (m=2114 / f=304). Ratio: 4.0:1 m= 87.43% f= 12.57%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: Simons Simplex Collection – repository of genetic sample from simplex families.	*Confirmed With*: ADI-R ADOS	*ABC*: Associated behaviour. *ADI-R:* Autism symptoms. *ADOS:* Autism symptoms. *CBCL:* Associated behaviour. *CTOPP:* Language. *DCDQ*: Motor function. *Grooved Pegboard test*: Motor function. *PPVT-4:* Language. *RBS-R*: Autism symptoms. *SRS:* Autism symptoms. *VABS2*: Adaptive behaviour.	Points of Data Collection Single. Data Sources *ADI-R*: INT *ADOS*: INT/TB/OBS/RS *ABC*: BRS *CBCL*: CHK *CTOPP*: INT/TB/OBS *DCDQ*: RS *Grooved Pegboard test*: NA/CA/TB/OBS *PPVT-4*: TB/OBS *RBS-R*: RS *SRS*: BRS *VABS2*: INT Data Context (Environment) Not Specified.	*Parent Report:* ABC: Informant Not Specified *Clinician Administered (Participant):* ADOS: Examiner Not Specified. CTOPP: Examiner Not Specified. PPVT-4: Examiner Not Specified. Grooved Pegboard test: Examiner Not Specified. DCDQ: Examiner Not Specified. *Clinician Administered (Parent):* ADI-R: Examiner Not Specified. CBCL: Informant Not Specified. RBS-R: Informant Not Specified. SRS: Informant Not Specified.
Park et al, 2012	*Aged:* 4-15 yrs. *Diagnosis*: DSM-IV-TR ASD	*ASD*: 111 (m=91 / f=20). Ratio: 4.6:1 m= 81.98% f= 18.02% *SIB*: 98 (m=47 / f=51). Ratio: 1.1:1* m= 47.96% f= 52.04% *TD*: 51 (m=26 / f=25). Ratio: 1.04:1 m= 50.98% f= 49.02%	*Matched*: Age & IQ. *Exclusion criteria*: Psychiatric Diagnosis.	*Sourced from*: *ASD*: Autism Genetic Study Consortium. *TD*: Community Volunteers	*Confirmed With*: DSM-IV-TR checklist SCQ| ASDS ADI-R	*ADI-R*: Confirm diagnosis, Autism symptoms. *ASDS:* Screening, Autism symptoms. *AQ*: Cognitive Style. *CBCL*: Co-occurring Psychopathology. *DSM-IV-TR Checklist*: Confirm diagnosis. *EQ*: Cognitive Style. *Leiter International Performance Scale*: IQ. *SCQ*: Screening, Autism symptoms. *SQ*: Cognitive Style.	Points of Data Collection Single. Data Sources *ADI-R*: INT *ASDS*: RS *AQ*: QU *CBCL*: CHK *DSM-IV-TR*: CHK *EQ*: QU *Leiter Scale*: INT/TB/OBS *SCQ*: RS *SQ*: QU Data Context (Environment) Not Specified.	*Parent Report:* AQ-C EQ-C SQ-C *Clinician Administered (Participant):* ADI-R: Examiner Not Specified. ASDS: Examiner Not Specified. DSM-IV-TR: Examiner Not Specified. SCQ: Examiner Not Specified. Leiter Scale: Examiner Not Specified. CBCL: Informant Not Specified.

Kopp & Gillberg, 2011	*Aged*: 6 – 16 yrs. *Diagnosis*: DSM-IV ASD: M20; F27 = 47 ADHD: M35; F37 = 72 Other neuropsychiatric conditions: M7; F7 = 14	*Clinic ASD*: 47 (m= 20/ f=27). Ratio: 1.4:1 m= 42.55% f= 57.45% *(Note: ADHD samples not included)* *Community*: 58 (m=0 / f=58). Ratio: 0:1 m= 0% f=100%	*Clinic Matched*: Age, IQ, & main diagnosis. Clinic exclusions: previously diagnosed LD (FSIQ ≥70). *Community Matched*: Age. *Community exclusions*: known medical illness, neuropsychiatric disorder, major academic problems, siblings assessed for a neuropsychiatric disorder, & parents with adequate language skills.	*Sourced from*: *Clinic*: Neuropsychiatric clinic (referrals) *Community*: local paediatric outpatient centre	*Confirmed With*: Parent interview schedule covering all child psychiatric and developmental diagnose of the DSM-IV	*ASSQ*: Autism symptoms. *ASSQ-GIRL*: Autism symptoms. *ASSQ-REV*: Autism symptoms. *Conners' Scale*: ADHD & problem behaviour. *DSM-IV Criteria*: Confirm diagnosis.	Points of Data Collection Single. Data Sources *ASSQ*: RS *ASSQ-GIRL*: RS *ASSQ-REV*: RS *Conners' Scale*: RS *DSM-IV*: CHK Data Context (Environment) Not Specified.	*Parent Report:* Conners' Scale ASSQ ASSQ-GIRL ASSQ-REV *Clinician Administered (Participant):* DSM-IV: Examiner Not Specified.
Holtmann et al, 2007	*Aged*: 5–20 yrs. *Diagnosis*: ICD-10 Autism: M20; F20 = 40 Atypical Autism: M2; F2 = 4 AS: M1; F1 = 2	46 (m=23 / f=23). Ratio: 1:1 m= 50.00% f= 50.00%	*Matched*: Age, ASD diagnosis & IQ. *Exclusion criteria*:	*Sourced from*: Department of Developmental Child and Adolescent Psychiatry or from within an international project on the molecular genetics of autism.	*Confirmed With*: ADI-R ADOS CBCL	*ADI-R*: Autism symptoms. *ADOS*: Autism symptoms. *CBCL*: Behavioural problems.	Points of Data Collection Single. Data Sources *ADI-R*: INT *ADOS*: INT/TB/OBS/RS *CBCL*: CHK Data Context (Environment) Not Specified.	*Parent Report:* CBCL *Clinician Administered (Participant):* ADOS: Examiner Not Specified. *Clinician Administered (Parent):* ADI-R: Examiner Not Specified.
Hess et al, 2010	*Aged*: 4-16 yrs. *Diagnosis*: DSM-IV-TR/ICD-10 AD AS PDD-NOS	*ASD*: 70 (m=44 / f=26). Ratio: 1.7:1 m= 62.86% f= 37.14% *TD*: 59 (m=27 / f=32). Ratio: 1.2:1* m= 45.76% f= 54.24%	*Matched*: Unknown. *Exclusion criteria*: Unknown.	*Sourced from*: community organisations, schools, and outpatient clinics.	*Confirmed With*: Unknown.	*ASD-CC*: Comorbid psychiatric symptoms. *DSM-IV*-TR: Confirm diagnosis, Autism symptoms. ICD-*10 Checklist*: Confirm diagnosis, Autism symptoms.	Points of Data Collection Single. Data Sources *ASD-CC*: RS *DSM-IV-TR*: CHK *ICD-10 Checklist*: CHK Data Context (Environment) Not Specified.	*Parent Report:* ASD-CC *Parent, Caretaker, Clinician Report:* DSM-IV-TR/ICD-10: Examiner Not Specified.

Kozlowski et al, 2011	*Aged*: 2-17 yrs. *Diagnosis*: DSM-IV-TR/ICD-10 (ASD-DC) An ASD	*ASD*: 187 (m=112 / f=75). Ratio: 1.5:1 m= 59.89% f= 40.11% *TD*: 204 (m=112 / f=92). Ratio: 1.2:1 m= 54.90% f= 45.10%	*Matched*: Unknown. *TD Exclusions*: Comorbid Axis 1 or Axis 2 conditions.	*Sourced from*: Outpatient clinics, schools, and parent advocacy and support groups.	*Confirmed With*: ASD-DC	*ASD*-DC: Autism symptoms, comorbid psychopathology, & co-occurring challenging behaviours. ASD-*BPC*: Autism symptoms, comorbid psychopathology, & co-occurring challenging behaviours.	Points of Data Collection Single. Data Sources *ASD-DC*: RS *ASD-BPC*: RS Data Context (Environment) Either home or clinic.	*Parent Report:* ASD-DC: Specified as "Informant". ASD-BPC: Specified as "Informant".
Rivet & Matson, 2011b (Study 2)	*Aged;* 3–17 yrs. *Diagnosis*: DSM-IV-TR/ICD-10 An ASD	*ASD*: 74 (m=37 / f=37). Ratio: 1:1 m= 50.00% f= 50.00% *TD*: 74 (m=37 / f=37). Ratio:1:1 m= 50.00% f= 50.00%	*Matched*: Best extent possible on demographic variables (e.g., age, ID, sensory impairments, epilepsy). *Exclusion criteria*: Sex chromosome disorders. *TD exclusions*: axis I disorders	*Sourced from*: Outpatient clinics, schools, and parent advocacy and support groups.	*Confirmed With*: DSM-IV-TR checklist ICD-10 checklist	*ASD-DC*: Autism symptoms. *DSM-IV*-TR: Autism symptoms. ICD-*10 Checklist*: Autism symptoms.	Points of Data Collection Single. Data Sources *ASD-DC*: RS *DSM-IV-TR*: CHK *ICD-10*: CHK Data Context (Environment) Not Specified.	*Parent/Caregiver Report:* ASD-DC DSM-IV-TR/ICD-10
McLennan et al, 1993	*Aged*: 6-36 yrs. *Diagnosis*: DSM-III ASD	43 (m=21 / f=21). Ratio: 1:1 m= 50.00% f=50.00%	*Matched*: Age, Diagnosis and IQ. *Exclusion criteria*: Unknown.	*Sourced from*: records of two Autism and PDD Clinics, Psychiatric Services, special education programs, and social services	*Confirmed With*: DSM-III checklist based on case notes prior to recruitment.	*ADI*: Autism symptoms. DSM-III checklist: Confirm diagnosis (based on case notes).	Points of Data Collection Single. Data Sources *ADI*: INT *DSM-III*: CHK Data Context (Environment) Not Specified.	*Clinician Administered (Participant):* DSM-III: Examiner Not Specified. *Clinician Administered (Parent):* ADI: 3 researchers.

Appendix B

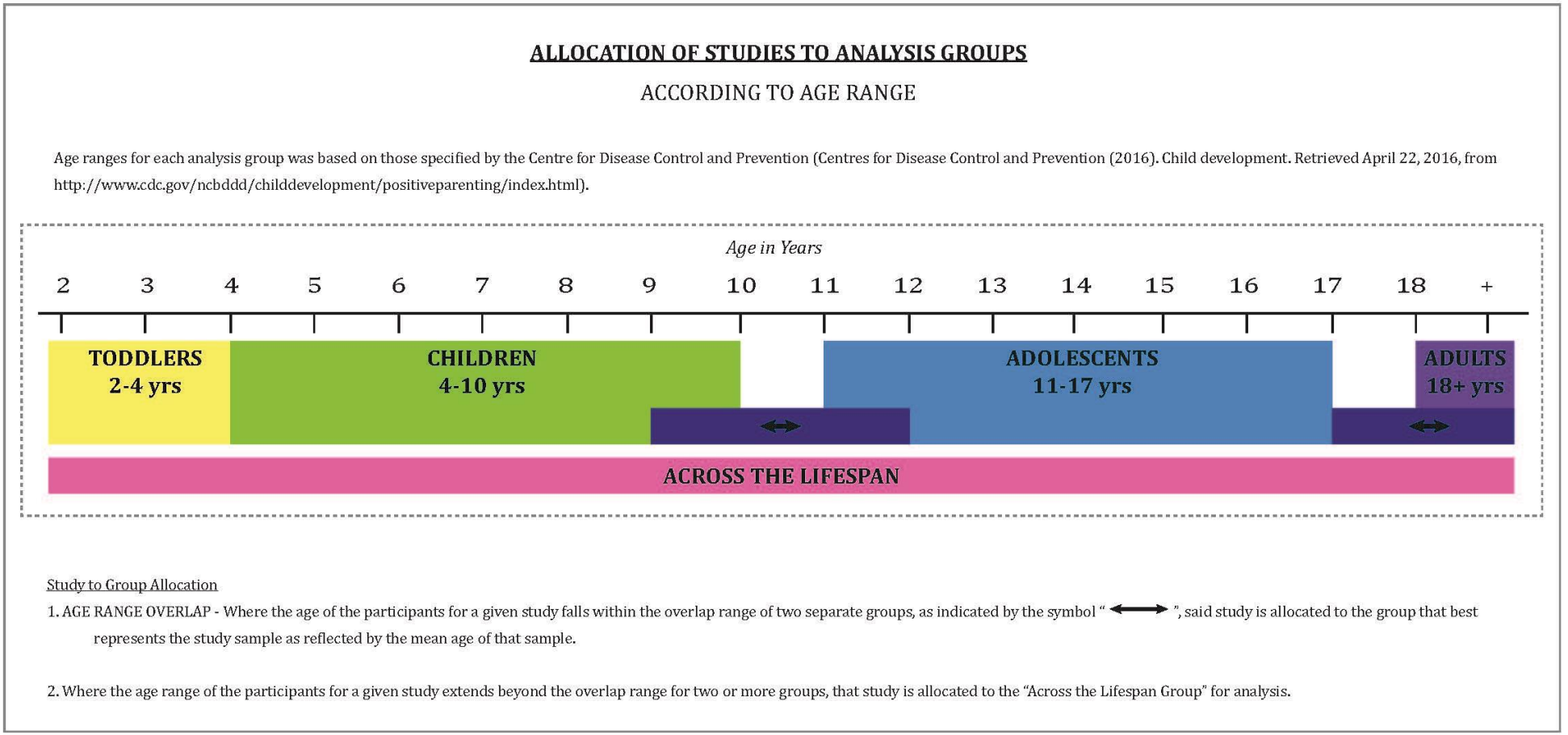

Figure A 1: Visual representation of the allocation of studies to analysis groups according to the age ranges employed within each study included in this review in order to identify the most common ages represented in the research. Developed by Grime, S. I.